ALLERGIC CONTACT DERMATITIS TO NICKEL: A STUDY OF ANTIGEN PRESENTATION *IN VITRO*

by

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DECLARATION

I declare that the work described in this thesis is my own and that the thesis has been composed by myself

K.M. Everness

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List of Abbreviations Used in Text

ACD	Allergic contact dermatitis
AET	Aminoethylisothiuronium
AMLR	Autologous mixed lymphocyte reaction
APC	Antigen presenting cell
C'	Complement
Con A	Concanavalin A
DH	Delayed hypersensitivity
DMG	Dimethylglyoxime
DNCB	2,4-dinitrochlorobenzene
ETAF	Epidermal cell-derived thymocyte activating factor
FCS	Foetal calf serum
HLA	Human leucocyte antigen
HSA	Human serum albumin
HSV	Herpes simplex virus
IL	Interleukin
LC	Langerhans cell
LTT	Lymphocyte transformation test
2-ME	2-Mercaptoethanol
MHC	Major histocompatibility complex
MIF	Migration inhibition factor
MLR	Mixed lymphocyte reaction
MMIT	Macrophage migration inhibition test
$NiSO_4$	Nickel sulphate
NRS	Normal rabbit serum
PBM	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PHA	Phytohaemagglutinin
PPD	Purified protein derivative of tuberculin
SALT	Skin-associated lymphoid tissues
SCW	Streptococcal cell wall antigen
SI	Stimulation index
Sj	Schistosoma japonicum antigen
SRBC	Sheep red blood cells
TCM	Tissue culture medium

ABSTRACT

Diagnosis of nickel allergy is often based on *in vivo* patch testing. This thesis describes a lymphocyte transformation test (LTT) in which soluble nickel sulphate ($NiSO_4$) was used to stimulate peripheral blood mononuclear cells (PBM) *in vitro* from nickel sensitive individuals.

Sixty-six patch test positive patients, together with 46 non-sensitive controls were studied using 0-20μg/ml $NiSO_4$ in a 6 day LTT; cells from 55 patients and 43 controls were also studied in a 7 day assay. Cells from 61/66 patients (92%) gave a stimulation index (SI) of $\geqslant 3$ with 5μg/ml of nickel sulphate in a 6 and/or 7 day assay. Cells from all 43 controls gave an SI <3. Cells from patients and controls were stimulated equally by the mitogen Con A. This indicates that using appropriate culture times and concentrations of antigen, the LTT is both sensitive and specific in confirming contact sensitisation to nickel.

The 6 day LTT was modified to study the accessory cell function of plastic-adherent blood cells and epidermal cells. PBM were subjected to plastic-adherence, incubation on nylon wool columns and finally adherence to glass to obtain an enriched population of T-cells which normally contained $<0.5\%$ MHC II^+ cells and was incapable of proliferation in the presence of either $NiSO_4$ or Con A. Addition of plastic-adherent blood cells (containing 55% MHC II^+ cells) reconstituted the antigen-specific response in 16/18 experiments using cells from nickel-sensitive patients. In 15/16 experiments, enriched T-cells from non-sensitised controls proliferated in the presence of Con A but not $NiSO_4$ when they were similarly reconstituted with plastic-adherent blood cells.

Epidermal cells, obtained from suction blisters, and containing 2% MHC II^+ cells also reconstituted or even enhanced the response of enriched T-cells from nickel sensitive patients to Con

A or $NiSO_4$. Epidermal cells from four controls reconstituted the response of enriched T-cells to Con A only.

The role of MHC II molecules in controlling the response of T-cells to $NiSO_4$ and to Con A was studied using the 6 day LTT and a number of monoclonal antibodies against HLA-DP, -DQ and -DR. Antibodies against HLA-DR inhibited consistently the antigen-specific response (82-96% inhibition) and normally inhibited (40-98%) the response to Con A, both of PBM and enriched T-cells reconstituted with plastic-adherent blood cells. At equivalent antibody concentrations, an antibody against HLA-DP also inhibited the response of PBM to nickel sulphate, but to a lesser extent (32-58%). Antibodies against HLA-DQ and $beta_2$microglobulin (a control antibody) had no effect on the proliferation of PBM in response to either $NiSO_4$ or Con A.

In conclusion, these studies have shown that the LTT, using 5µg/ml $NiSO_4$ in 6 and 7 day assays is an excellent method for detecting nickel sensitivity, showing good correlation with the *in vivo* patch test. The method has been adapted to demonstrate the capacity of blood and epidermal cells to act as accessory cells, and to assess the role of MHC class II molecules on the surface of these cells in controlling the proliferation of T-cells in response to both an antigenic and a mitogenic stimulus.

INTRODUCTION

The complex phenomenon of contact sensitivity or allergic contact dermatitis (ACD) has been investigated for more than a century. In 1840, Fuchs observed that only certain individuals reacted against particular chemicals and his observations were followed by skin testing to common chemicals including mercury salts and soaps (Hebra, 1869; cited by Dupuis and Benezra, 1982). However, Jadassohn is credited with establishing the concept of ACD after reporting that the application of iodoform to the normal skin of five sensitised (allergic) subjects reproduced their dermatitis (1896).

Preliminary studies on ACD were carried out on human volunteers experimentally sensitised to extracts of Primula leaves (Low, 1924; Bloch and Steiner-Wourlisch, 1926), p-phenylenediamine (Mayer, 1928) and 2,4-dinitrochlorobenzene (DNCB) (Wedroff, 1927, 1932; cited by Dupuis and Benezra, 1982). The discovery of a suitable experimental animal model (the guinea pig) (Bloch and Steiner-Wourlisch, 1930; Jadassohn, 1930; cited by Dupuis and Benezra, 1982) has permitted extensive research into the immunological mechanisms of ACD. Contact sensitivity has since been induced in several animal species including mice, rats, dogs, chickens and monkeys (Polak, 1980).

Work carried out by Landsteiner and his group greatly contributed to the research into the mechanism of ACD. Firstly, they established the importance of chemical structure in contact sensitivity by demonstrating that low molecular weight substances called haptens (MW 400-900 Da) require conjugation with protein carriers before stimulation of the immune system can take place (Landsteiner and Jacobs, 1935). Secondly, they confirmed the existence of two types of immune reaction (humoral and cell-mediated) by transferring contact sensitivity to picryl chloride from a hypersensitive to a normal guinea pig using living lymphoid cells, but not using serum (Landsteiner and Chase, 1942).

In 1961, it was discovered that lymphocytes comprise two functionally different populations; B-cells (bursa-equivalent lymphocytes) which are the precursors of antibody producing plasma cells and T-cells (thymus-dependent lymphocytes) which are responsible for cell mediated immunity (Miller, 1961).

It is now well established that ACD is a delayed hypersensitivity (type IV) reaction mediated by T-cells against haptens which are absorbed through the skin.

The reaction can be divided into two phases, induction and elicitation. During the induction phase hapten binds to protein (possibly within the skin), forming an antigen which can be recognised by hapten-specific virgin T-cells. These cells become sensitised. They proliferate in the draining lymph nodes (Turk and Oort, 1970) to produce memory and effector cells (Turk and Stone, 1963; cited by Polak, 1980).

The elicitation phase occurs when a sensitised individual subsequently contacts the same hapten. The hapten again binds to protein producing an antigen which can be recognised by the hapten-specific effector T-cells. These cells secrete a number of soluble substances (lymphokines) which attract and activate non-specific mononuclear cells to the site of hapten application, amplifying the response and causing a local inflammatory reaction.

During the last ten years, the mechanisms involved in antigen-(including hapten-) specific T-cell activation have been further clarified, and the next section reviews this work in some detail.

ANTIGEN-SPECIFIC T-CELL ACTIVATION

Established techniques to enrich T-cells and to produce T-cell clones have been used to show that mitogen- or antigen-specific T-cell activation requires the presence of another cell type (an accessory cell). Requirements for T-cell activation will be discussed as follows:-

T-cell subtypes and markers

Accessory cells. (1) cells with accessory function

(2) requirements:- MHC restriction

Antigen processing

Interleukin-1 production

T-cell receptor and antigen-specific T-cell activation.

T-Cell Subtypes and Markers

All human T-cells express receptors for sheep red blood cells (CD2) and the CD3 antigen; however, T-cells can be divided into 4 functional subgroups:-

T_h- helper cells - stimulate the immune response

T_s- suppressor cells - depress the immune response

T_c- cytotoxic cells - kill virally infected or foreign cells

T_{dh} - delayed hypersensitivity cells - secrete lymphokines (Nash and Gell, 1981)

In addition, 2 phenotypic populations exist:-

$CD4^+$ (T_h, T_{dh})

$CD8^+$ (T_s, T_c);

although the phenotypes $CD4^+$ and $CD8^+$ may overlap between the functional groups (Spits et al., 1983).

There is no good in vitro model of delayed hypersensitivity (DH), but DH function is normally correlated with results obtained using a lymphocyte transformation test (LTT), which measures cellular incorporation of radioactive thymidine into newly synthesised DNA, which precedes the proliferation of T-cells in response to the presence of hapten or antigen.

Accessory Cells

Many cell types have been shown to act as accessory cells in lymphocyte transformation. Examples are shown in Table 1.1.

The earliest experiments on accessory cell function were carried out using macrophages; therefore, the macrophage will often be used as an example of an accessory cell during this thesis. Furthermore, initial experiments were carried out primarily on mice and guinea pigs and not on humans.

Table 1.1 Accessory Cells

Cells	Key Reference
macrophages	Seeger and Oppenheim (1970)
lymph node follicular dendritic cells	Klaus et al. (1980)
spleen dendritic cells	Sunshine et al.(1983)
blood dendritic cells	Van Voorhis et al.(1983)
vascular endothelial cells	Hirschberg et al.(1980)
B-cells	Ashwell et al.(1984)
activated T-cells	Engleman et al.(1980)
veiled cells	Balfour et al.(1981)
epidermal cells	Stingl et al.(1978)

MHC Restriction

In 1973, Rosenthal and Shevach showed that antigen-specific T-cell proliferation required histocompatibility between the accessory cells (macrophages) and the T-cells. It is now firmly established that most T-cells will recognise antigen only when it is associated on the cell surface with a self molecule encoded by the Major Histocompatibility Complex (MHC); a phenomenon known as MHC restriction. In man, the MHC codes for human leucocyte

antigens (HLA). The functionally different T-cell subclasses vary with respect to the type of molecule they recognise:- T_c are restricted by class I molecules (Zinkernagel and Doherty, 1975); T_h and T_{dh} are restricted by class II molecules (Thomas et al., 1977); however, these subpopulations probably overlap (Braciale, 1987).

The Major Histocompatibility Complex

In man the MHC represents approximately one thousandth of the genome and is situated on the short arm of chromosome 6. It was named the MHC because the HLA molecules encoded were first recognised as classical transplantation antigens on the cell surface. There is no allelic exclusion of MHC antigens and they are expressed co-dominantly. The structure of the complex is summarised in fig 1.1 and comprises several loci.

HLA-A -B -C : code for the MHC class I molecules

HLA-D : codes for the MHC class II molecules

In addition, there are loci coding for MHC class III molecules which are components of the complement cascade. Only class I and class II molecules are involved in T-cell antigen recognition and will be discussed below.

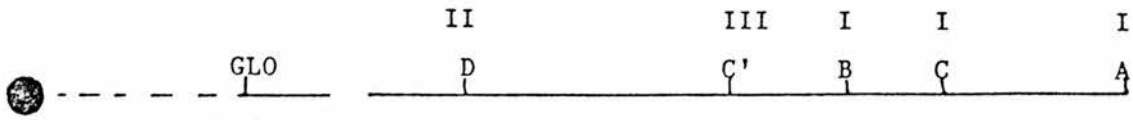
Class I

Class I HLA antigens (fig 1.3) are found on almost all nucleated cells and comprise a 45kDa transmembrane glycoprotein (alpha chain) in non-covalent association with an invariant 12kDa protein, $beta_2$ microglobulin. Alpha chains are encoded in the MHC, micro-globulin at a locus on chromosome 15. Expression of the alpha chain requires the association with $beta_2$ microglobulin.

Class II

Class II HLA antigens (fig 1.4) have a more limited tissue distribution than class I molecules. They are expressed on cells such as macrophages, B-lymphocytes and dendritic cells, which are cells involved in interactions with T-lymphocytes during immune responses. Class II molecules consist of two transmembrane glycoproteins- a 33kDa alpha chain and a 28kDa beta chain. Before

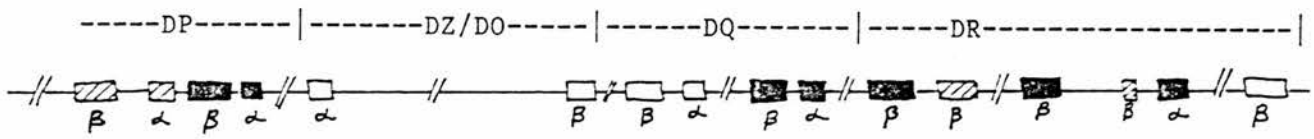
Fig 1.1 Map of the MHC



GLO = Glyoxalase

The HLA complex is situated on the short arm of chromosome 6.

Fig 1.2 Map of the human HLA-D Region

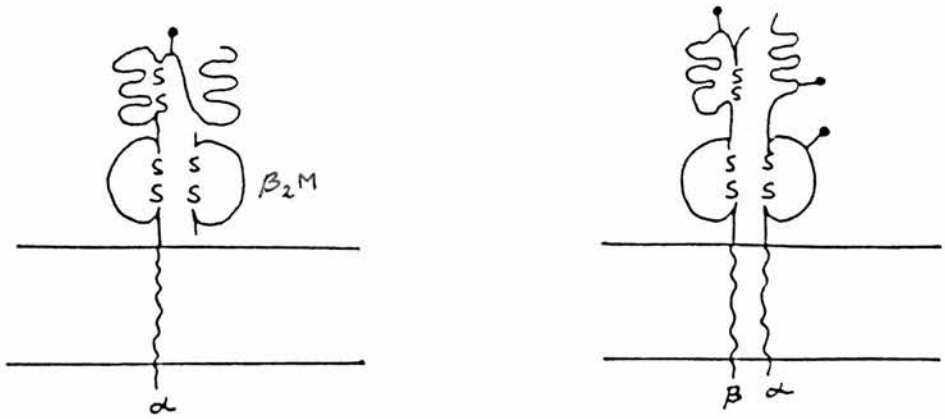


Expressed genes are depicted as filled boxes, pseudogenes by cross-hatching, and genes of undetermined status are blank.
From Trowsdale (1988)

Fig 1.3
Class I MHC Antigen

Fig 1.4
Class II MHC Antigen

S-S = Disulphide bonds between cysteine residues.
• = Carbohydrate side chain.
β_2M = $\beta_2$microglobulin.



insertion into the membrane, the class II molecules are associated with a third invariant gamma chain (Kvist et al., 1982).

The map of the HLA-D region (fig 1.2) is still provisional.

The only class II gene products presently known to be expressed on the cell surface are HLA-DP, -DQ, and -DR. Evidence exists that antigen forms a complex with the MHC II molecule (Babbitt et al., 1985); however, the degree of specificity with which antigen binds MHC II molecules is uncertain (Finnegan and Berzofsky, 1986). An antigen binding groove may be present in MHC II molecules (Bjorkman et al., 1987).

Experiments using antibodies directed against class II molecules indicate that HLA-DR is required for antigen-specific proliferation of T-cells, and preliminary evidence suggests that the expression of HLA-DQ may be important in suppressor cell activation (Festenstein and Ollier, 1987). The biological significance of HLA-DP awaits clarification.

Expression of MHC II antigens on antigen presenting cells (APC) is not constant. Substances released during contact sensitivity reactions can alter MHC II expression. Prostaglandins are important mediators of inflammation, and prostaglandins E1 and E2 diminish MHC II expression (Snyder et al., 1982). Conversely, activated T-cells release gamma-interferon (Schober et al., 1984) which has been shown to enhance (Berman et al., 1985) or induce (Basham et al., 1984) MHC II expression in different cell types.

However, the expression of class II molecules on a cell surface is not the only requirement for T-cell activation.

Antigen Processing

Most complex antigens require processing before presentation to T-cells. Antigen is taken up by phagocytic cells, endocytosed and then fragmented. The antigen is then recycled to the cell surface in a form capable of association with MHC II molecules (Allen and Unanue, 1984). The MHC II molecule may bind the antigens, stabilise them in the cell membrane and prevent proteolytic degradation (Werdelin, 1987). Some of the immunologically relevant antigen remains within the accessory cell (Ellner et al., 1977). Haptens, being low molecular weight substances, may not require antigen processing.

Interleukin 1 Production

Accessory cells must also be capable of producing a low molecular weight molecule, Interleukin 1 (IL-1), which is antigen-non-specific (Aarden et al., 1979). Interleukin 1 is produced by most cell types (Oppenheim et al., 1986) and acts as a maturation signal, preparing T-cells to respond to antigens or secondary mediator signals (Mizel, 1982). Unstimulated macrophages release little IL-1; macrophage IL-1 production is probably activated by the presence of T-cells plus antigen or mitogen. Interleukin 1 release may be stimulated further by T-cells after their maturation, thus amplifying the initial phase of the immune response. Mitogens (which non-specifically cross-link T-cell receptors) can activate T-cells in the absence of accessory cells if IL-1 is present (Farrar et al., 1980); however, T-cell stimulation by IL-1 in antigen-specific responses is totally dependent on the presence of accessory cells (Mizel and Ben-Zvi, 1980).

MHC II Antigen Expression In Disease States

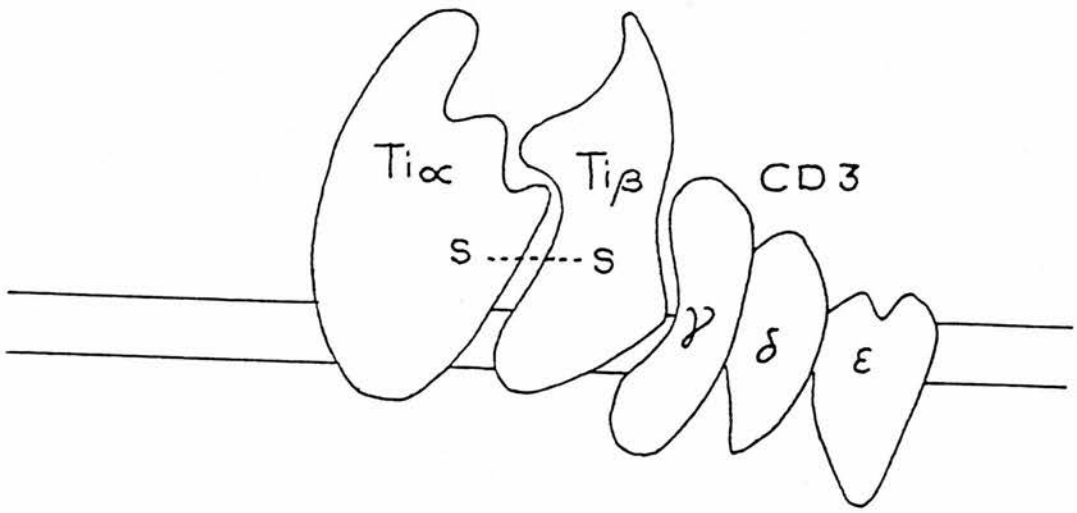
Within the last 7 years it has been demonstrated that several types of epithelial cell which do not normally express MHC II

antigens, become MHC II positive at sites of inflammation caused by certain disease states, eg thyroid cells in autoimmune thyroiditis (Hanafusa et al., 1983) and keratinocytes in graft versus host disease (Mason et al., 1981). Thyroid follicular epithelial cells expressing class II molecules in thyroiditis possess the ability to present peptide antigen to cloned T-cells, and the presence of these "aberrant" MHC II molecules may be important in perpetuating or even initiating auto-immune disease (Londei et al., 1984). Keratinocytes express MHC II antigens in many dermatoses including graft versus host disease, mycosis fungoides, lichen planus and contact dermatitis, in which there are also many activated T-cells present. Cultured keratinocytes have been shown to express MHC II antigens when incubated with gamma-interferon (Basham et al., 1984) and it is probable that keratinocyte MHC II expression is mediated by gamma-interferon released by T-cell infiltrates (Nickoloff et al., 1985).

Antigen-Specific T-Cell Activation

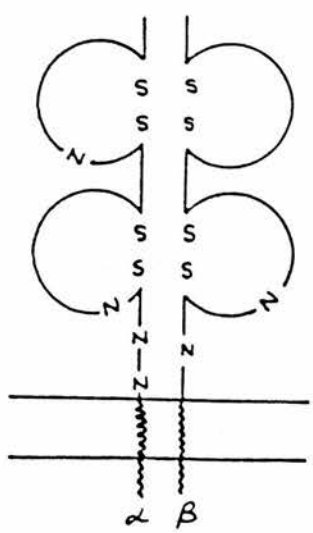
The structure of the T-cell receptor has been established in studies using antigen-specific T-cell clones and monoclonal antibodies directed against their membrane molecules (Acuto and Reinherz, 1985). It is now known that the T-cell receptor exists as a surface complex comprising a 90kDa di-sulphide-linked heterodimer (Ti molecule) and three monomorphic molecules (gamma, delta and epsilon) which make up the CD3 complex (fig 1.5). Furthermore, the CD4 antigen, a marker specific for T_h and T_{dh} cells, may also be in physical association with the T-cell receptor complex (Saizawa et al., 1987). The Ti alpha and beta chains (fig 1.6) are immunoglobulin-like and confer variability to a T-cell; transfection experiments indicate that the alpha and beta genes are sufficient to code for MHC-restricted antigen recognition (Dembic et al., 1986). More recently, a second T-cell receptor comprising a gamma and delta chain has been identified on $CD4^-$ $CD8^-$ cells. However, the role of this receptor (discussed by Owen, 1988) is unknown.

Fig 1.5 T-Cell Receptor Complex (Ti-CD3)



Ti α and β subunits are held together by S-S bonds and are associated with the gamma subunit of the CD3 molecule.
From Alcover et al. (1987).

Fig 1.6 Structure of the Ti alpha and beta chains



From Owen and Crumpton (1987)

It is thought that the following events occur during T-cell activation (fig 1.7)

(a) The Ti molecules recognise MHC II and antigen. The CD4 antigen may bind to a non-polymorphic portion of MHC II and stabilise the reaction. Interleukin 1 is secreted by the APC.

(b) The CD3 complex sends an activation signal across the lipid bilayer; ion channels in the plasma membrane are opened and calcium enters down its concentration gradient (Alcover et al., 1987). The alteration in the intra-cellular cations effects gene transcription.

(c) The number of surface antigen-specific receptors decreases.

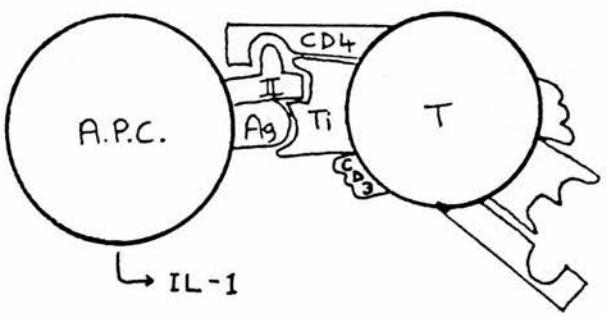
(c and d) Activation stimulates endogenous Interleukin 2 (IL-2) production and secretion, and the expression of IL-2 receptors on the same clones. Interleukin 2 also stimulates the release of T-cell gamma-interferon which enhances MHC II molecule expression on the APC.

(e) Once a critical number of receptors have bound IL-2, DNA synthesis and mitosis of the cell occur.

(f) After antigenic stimulation ceases, T-cells re-express the Ti-CD3 complex, and the number and affinity of IL-2 receptors decrease.

Fig 1.7 Antigen-Specific T-Cell Activation

(a)

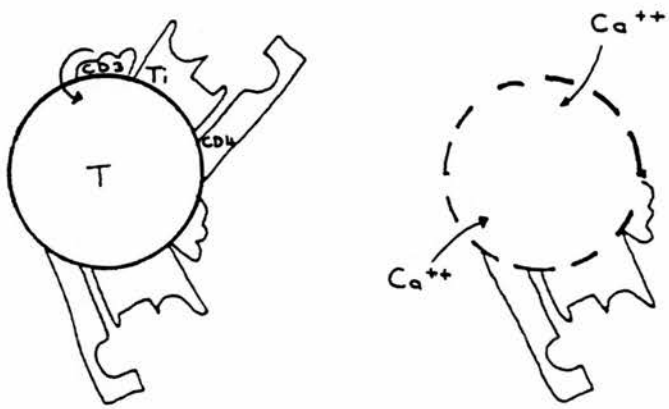


Ti recognises MHC II and antigen.

CD4 binds MHC II.

IL-1 is secreted.

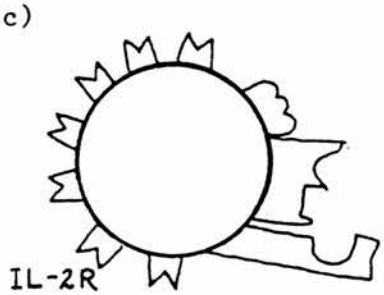
(b)



CD3 sends activation signal across membrane.

Ca^{++} enters cell.

(c)

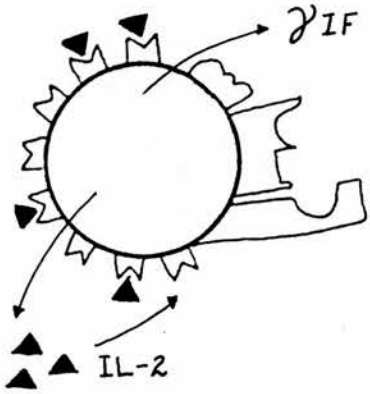


Antigen-specific receptors decrease.

IL-2 receptors (IL-2R) expressed.

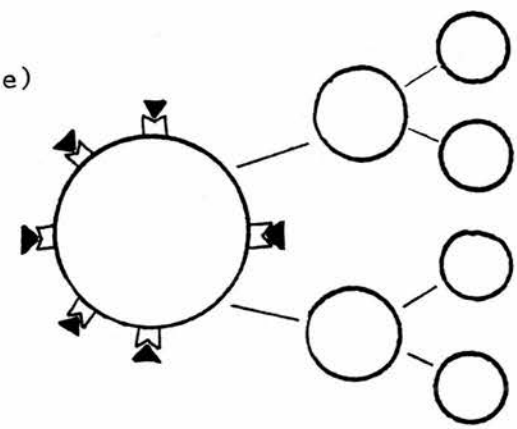
Fig 1.7 (continued) Antigen-Specific T-Cell Activation

(d)



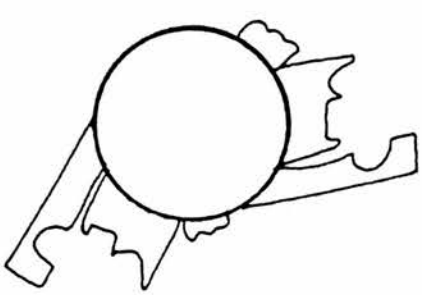
Cells secrete IL-2 and gamma interferon.

(e)



IL-2 binding permits DNA synthesis and mitosis resulting in the clonal expansion of T-cells.

(f)



IL-2 receptors decrease.

Ti-CD3 increases.

MITOGEN-INDUCED T-CELL PROLIFERATION

Two plant lectins (proteins which specifically bind certain sugars), Concanavalin A (Con A) and phytohaemagglutinin (PHA), are T-cell mitogens and are commonly used in *in vitro* transformation assays. However, the mechanisms of antigen-specific and mitogen-induced T-cell activation may not be identical.

Some experiments indicate that accessory cells are unnecessary for T-cell activation by these mitogens. Investigators have shown that the presence of serum in culture may replace some accessory cell function (Persson *et al.*, 1978) and it is possible that the serum contains soluble factors released by monocytes (including MHC II molecules and IL-1). The reducing agent 2-mercaptoethanol (2-ME) has also been shown to stimulate Con A-induced proliferation of enriched T-cells (Roosnek *et al.*, 1985). However, it has been suggested that the relationship between monocytes and thiols is an *in vitro* phenomenon.

Conversely, many reports indicate that a mitogen-induced proliferative response is dependent on the presence of accessory cells (Hedfors *et al.*, 1975; Rosenstreich *et al.*, 1976; Habu and Raff, 1977). In the presence of mitogenic concentrations of lectin, cross-linking of cell membrane molecules occurs (Con A binds alpha-D mannose and alpha-D glucose) but the exact requirement for accessory cells in Con A-induced T-cell proliferation is unknown.

Cell-cell contact may be a prerequisite for T-cell activation. Mitogenic amounts of lectin do not cluster cells and it has been suggested that accessory cells may be required to induce aggregation (Roosnek *et al.*, 1985). However, it is unlikely that this is the reason for the accessory cell requirement in mitogen-induced proliferation because neuraminidase treatment of cells (which removes the cells' negative charges, permitting agglutination) does not mediate T-cell proliferation (Hunig, 1983).

Addition of IL-1 to enriched T-cells incubated with PHA does not induce T-cell activation (Chu et al., 1985). This indicates that the release of IL-1 by accessory cells is not the only factor required for T-cell activation.

Finally, Kimura and Ersson (1981) have shown that lectins are mitogenic only if they are capable of binding to MHC II molecules. They suggested that mitogen-induced T-cell proliferation is similar in principle and requirements to antigen-specific T-cell activation. The recognition of mitogen by T-cells (without the need for 'antigen processing' (Chu et al., 1985)) may therefore be very similar to hapten-specific T-cell activation which as previously mentioned is unlikely to require antigen processing by an antigen presenting cell.

THE SKIN IMMUNE SYSTEM

The skin is important in protection from the environment because it functions as a physico-mechanical barrier. Furthermore, the skin may play an important role in the immune system. In 1978, Streilein hypothesised the existence of skin-associated lymphoid tissues (SALT). Five years later, he summarised the evidence for SALT as follows:-

(1) Specialised antigen presenting cells are found in the epidermis (Langerhans cells).

(2) Antigen recognition by lymphocytes can take place in the skin.

(3) Some recirculating T-cells preferentially infiltrate the skin (especially the epidermis).

(4) Draining lymph nodes accept immunological signals derived from the skin.

(5) Cutaneous immunity can be regulated by signals released by skin cells.

(Streilein, 1983).

It is now known that a number of immunologically relevant cells may be found in the skin (Table 1.2).

Table 1.2 Skin Cells With or Without Immunological Relevance

Skin Immune System Cells	Non-Immune Skin Cells
Keratinocytes	Melanocytes
Langerhans cells	Merkel cells
Indeterminate cells	Fibroblasts
Veiled cells	Eccrine gland cells
Mononuclear phagocytes	Duct cells
Neutrophils	Myoepithelial cells
Mast cells	Apocrine secretory cells
Vascular endothelial cells	Sebocytes
Lymphatic endothelial cells	Neural receptor cells
T-cells	Smooth muscle cells

(Bos and Kapsenberg, 1986)

Dendritic Cells Important In the Human Immune System

Dendritic antigen presenting cells in the human skin include Langerhans cells (LC) and indeterminate cells (which may be immature LC). In mice, two other epidermal cell types, the Thy-1^+cell (Tschachler et al., 1983; Bergstresser et al., 1983) and an ultraviolet radiation-resistant cell (Granstein et al., 1984) have been described in the epidermis. These two murine cell types may be capable of down-regulating the immune response (Bigby et al., 1987; Granstein et al., 1984). Equivalent cell types have not yet been found in man.

Epidermal Antigen Presenting Cells

Before the discovery that gamma-interferon induced keratinocyte MHC II antigen expression, it was assumed that only one epidermal cell type, the Langerhans cell, was capable of antigen presentation.

Langerhans Cells

In 1868 Paul Langerhans first described a population of dendritic epidermal cells after staining skin with gold chloride to

study nerve cells. Langerhans cells are found in most squamous epithelia and comprise approximately 3% of epidermal cells. However, their number varies with the anatomical site (Horton et al., 1984). Langerhans cells can be stained with osmium and DOPA and contain adenosine triphosphatase, alkaline phosphatase and cholinesterase (Rowden, 1981). In 1981, Poppema et al. showed that LCs express the CD1 marker. Using electron microscopy, LCs were shown to possess a distinctive cytoplasmic granule (the Birbeck granule) which, in thin sections, is rod-shaped but may have a vesicle at one end, giving the appearance of a tennis racquet (Birbeck et al., 1961).

An immunological function for the LC was suggested after the discovery of surface receptors for IgG Fc and complement C3 (Stingl et al., 1977). Studies carried out on lymph nodes draining sites of allergen application have suggested that LCs carry cutaneously applied allergen to the regional lymph nodes for presentation to T-cells (Godfrey and Baer, 1971; Botham et al., 1987). The expression of MHC II molecules (Rowden et al., 1977) together with an ability to produce IL-1 (Sauder et al., 1984) make it likely that LCs are antigen presenting cells.

In vitro assays have assessed the ability of epidermal cells to present antigen and mitogen to T-cells. Both crude epidermal cell suspensions (Stingl et al., 1978; Braathen and Thorsby, 1980) and enriched LCs (Bjercke et al., 1984) have accessory cell function.

Keratinocytes

Keratinocytes (90-95% of the epidermal cell population) have recently been shown to express MHC II antigens in certain disease states and in the presence of gamma-interferon (Basham et al., 1984). Furthermore, cultured keratinocytes produce soluble factors including epidermal cell-derived thymocyte activating factor (ETAF) which is a molecule equivalent to IL-1 (Sauder et al., 1982). The ability of MHC II positive keratinocytes to present antigens and mitogens has been studied. Nickoloff et al. (1986) showed that

HLA-DR$^+$ keratinocytes permitted T-cell activation but mitosis did not occur. They suggested that the keratinocytes were secreting an inhibitory molecule. Nishioka et al. (1987) failed to stimulate antigen-specific T-cells using MHC II positive keratinocytes; however, addition of IL-2 permits lymphocyte transformation (Morhenn and Nickoloff, 1987). Suction blisters removed from tuberculin-reactive skin (in which most keratinocytes are expressing HLA-DR) were more effective at presentation of PPD than suction blisters from control skin (Tjernlund and Scheynius, 1987). However, removal of LCs from these epidermal cell suspensions prevents accessory cell function (Scheynius et al., 1988), which may indicate synergy between LCs and HLA-DR$^+$ keratinocytes in antigen presentation. Further work is required before the role of MHC II positive keratinocytes in antigen presentation is defined.

THE INFLAMMATORY REACTION IN ACD

In ACD, activated T-effector cells release a variety of substances (lymphokines) which recruit inflammatory cells to the site, activate them and keep them at the site. The release of substances including lysosomal enzymes, prostaglandins (especially the vasodilator prostaglandin E_2) and complement factors by both the inflammatory infiltrate and the activated T-cells contribute to the clinical appearance of ACD.

ALLERGIC CONTACT DERMATITIS IN HUMANS

In its acute phase, a dermatitis (eczema) reaction is characterised by redness, oedema, papules, vesiculation, exudation, crusting and itch (fig 1.8). In chronic dermatitis, whether due to atopy (fig 1.9) or repeated contact with an allergen, the involved skin may become thickened, lichenified (an increase in skin markings), fissured and pigmented.

ACD may develop at any age but is uncommon in children. Some strongly allergenic substances induce allergy in the majority of people exposed to them (eg poison ivy); however, in general only a small number of people will be sensitised after contact with an allergenic chemical. At least 5 factors influence the induction of sensitisation to a chemical (Fregert, 1981).

1)sensitisation capacity of the chemical

2)skin damaging factors (eg skin disease, hydration, dryness)

3)environment of the skin surface (eg temperature, humidity)

4)exposure (eg concentration, occlusion)

5)genetic factors

The extent of dermatitis in an allergic subject is influenced by similar factors (Fisher, 1975)

Causative Agents

Thousands of substances have been described as contact allergens; however, only 20-30 substances cause most cases of ACD (Fregert and Bandmann, 1975). Aetiological agents may be identified by a procedure known as patch testing.

The value of patch testing patients suspected of having ACD was established by Bonnevie in 1939, twenty eight years after Bloch described a detailed procedure for applying allergens to the skin of normal and sensitised subjects (cited by Cronin, 1980). Patch testing is now a standardised procedure which allows comparison

Fig 1.8 Acute Vesicular Contact Dermatitis
(caused by a rubber glove)

Fig 1.9 Chronic (Atopic) Dermatitis

between different patients and different centres. The Dermatology Department at the Royal Infirmary, Edinburgh routinely uses the European Standard Battery (Table 1.3) recommended by the International Contact Dermatitis Research Group (ICDRG). When necessary, additional batteries related to occupation, clothing or hospital treatment are included. Patients may also be advised to bring items suspected of causing ACD for patch testing.

Table 1.3 European Standard Battery

Neomycin Sulphate	20.0%	Epoxy Resin	1.0%
Potassium Dichromate	0.5%	Fragrance mix*	8.0%
Wool alcohols	30.0%	Cobalt Chloride	1.0%
Mercapto rubber mixture*	1.0%	Balsam of Peru	25.0%
Benzocaine	5.0%	Thiuram rubber mixture*	1.0%
Nickel Sulphate	5.0%	Parabens mix*	15.0%
p-Phenylenediamine (PPD)	2.0%	PPD rubber mixture*	0.6%
Naphthyl rubber mixture*	1.0%	Turpentine peroxides	0.3%
Colophony	20.0%	Formaldehyde	2.5%
Caine mix*	3.0%	Primin	0.01%

* Contents of the mixtures are in Appendix I (pages 173-74).

Patch testing is described in the Materials and Methods section (page 43).

Marked variations exist in the prevalence of ACD to different substances in different populations, depending on the population studied (eg male/female) and the changing exposure of allergens. However, nickel compounds and rubber compounds remain two of the most common causes of ACD (Fisher, 1975; Vestey et al., 1986). It was because of patient availability that the studies reported in this thesis on in vitro requirements of antigen presentation in ACD were performed using nickel-sensitive patients.

NICKEL

Nickel was first isolated in 1751 and is a silver-white transition metal, which can be polished to give a bright gloss. The word 'nickel' is derived from 'kupfernickel' (devil's copper) originally used by German copper miners (1694) to describe niccolite (nickel arsenide) which visually resembles copper and when heated produces toxic fumes reminiscent of the Devil at work.

Nickel is the 24th most common element in the Earth's crust and has a widespread distribution in the environment. Certain plants and animals have an absolute requirement for nickel (Anke et al., 1984). Little is known about its biological functions in animals or man; however, nickel may be concerned with the maintenance of the structure and function of cellular membranes (Doll, 1984).

Man ingests 100-600µg of nickel per day; a deficiency state has never been identified. Foodstuffs possessing a high nickel content include legumes, leafy vegetables, mushrooms, chocolate, nuts and baking powder. Nickel may also be transferred to the diet by using nickel-containing kitchen equipment (Christensen and Moller, 1978). Less than 10% of ingested nickel is absorbed into the blood stream; 70% of this is then excreted via the kidneys; the remaining nickel is stored in body tissues and retained for approximately 200 days (Bennett, 1984). Five nickel-binding fractions have been described in serum (Silvennoinen-Kassinen et al., 1987) including

1) $Alpha_2$-macroglobin-bound nickel (nickeloplasmin)
2) Albumin-bound nickel (NH_2-terminal)
3) Amino acid (L-histidine) bound nickel

(Lucassen and Sarkar, 1979).

L-histidine has a greater affinity for nickel than HSA. Nickel exchange and transfer between these two binding fractions is mediated by the complex albumin-nickel-L-histidine, permitting

albumin-nickel to transfer the metal ion to a low molecular weight constituent of serum, which could then transport nickel into cells (Sarkar, 1984).

Nickel is a strong corrosion-resistant metal and therefore has a wide range of applications. However, although nickel is an industrially useful metal, under certain circumstances contact with this element is hazardous.

Carcinogenicity and Mutagenicity

An increased cancer risk among nickel workers was first suspected in 1932; however,the specific cancer inducing mechanism of nickel is not yet understood. Studies have shown that carcinogenicity of nickel compounds is inversely proportional to water solubility (Dewally, 1984) and directly proportional to cellular uptake (Costa and Mollenhauer, 1980). Nickel (probably as an ion) crosses the cell membrane, and after deposition in the nucleus and nucleolus, can form a complex with DNA (Ciccarelli and Wetterhahn, 1984).

Chromosomal damage in the leucocytes of nickel workers after short term culture has been described (Waksvik and Boysen, 1982) and white blood cells incubated with different nickel salts have undergone DNA strand breaks, sister chromatid exchange and chromosome aberrations (Saxholm, 1984).

Acute Toxicity

The chief industrial hazard of nickel is exposure to nickel carbonyl which can be fatal, with major lesions in the lungs and brain.

Embryotoxicity

Nickel compounds can penetrate the placenta and enter the foetus. Teratogenic effects result from a direct toxic effect on the foetus. Nickel may cause changes in the mitotic apparatus,

resulting in cell death at critical times of development (Leonard and Jacquet, 1984).

Immunotoxicity

Nickel compounds can adversely affect the immune system of experimental animals and it has been postulated that nickel-induced suppression may be related to nickel carcinogenicity (Smialowicz et al., 1985).

Immediate Type Hypersensitivity

The mechanism by which IgE antibodies initiate asthma is well understood and a number of allergens (eg grass pollens and the faecal pellets of the house dust mite) are recognised as causative agents. However, bronchial asthma induced by nickel dust aerosols is rare. The first case was described by McConnell et al. in 1973, and in 1982 a further two cases were reported (Malo et al., 1982; Block and Yeung, 1982). Patients can be diagnosed by inhalation challenge with nickel sulphate, positive skin prick tests and by a nickel-HSA radioallergosorbent test. The antigenic determinant in this allergy is nickel-HSA (Dolovich et al., 1984).

Cases of systemic immediate type hypersensitivity after iatrogenic nickel exposure have been reported. Patient symptoms included generalised itch and widespread urticaria (McKenzie et al., 1967) and swollen face, lips and hands accompanied by wheezing (Fisher et al., 1982).

Contact Dermatitis

Before 1930, nickel dermatitis was primarily an occupational disease, particularly in the plating industry. Cutaneous exposure to dissolved nickel may still cause dermatitis in certain occupations including electro-plating, hairdressing and domestic cleaning; however, the increased presence of nickel in the everyday environment (Table 1.4) has made nickel the most common sensitising agent.

Metallic nickel is usually the sensitiser; however, the water soluble salts, nickel chloride ($NiCl_2$) and nickel sulphate ($NiSO_4$) are also strong sensitisers (Cronin, 1980). The sensitisation potential of nickel salts was investigated in the 1960's using a 'maximisation' procedure; the skin was damaged by occluding the irritant sodium lauryl sulphate with the sensitiser for 5 periods of 48 hours (with 24 hour intervals between application). Using this method, Kligman (1966) attempted to sensitise 25 healthy subjects using 10% $NiSO_4$. Following challenge with 2.5% $NiSO_4$, responses were seen in 12 individuals. Nickel sulphate is now classified as a grade 3 sensitiser (Kligman, 1966) (grade 1 = weak sensitiser, 0-8% sensitisation (eg pure soap); grade 5 = extreme sensitiser, > 84% sensitisation (eg p-phenylenediamine). Once sensitisation to nickel has been acquired, it is probably permanent (Calnan, 1956).

Table 1.4	Examples of Articles Containing Nickel
Clothing	Zips, suspenders, bra straps, jeans buttons.
Jewellery	Earrings (pierced/clip on), necklaces, rings, watches, bracelets.
Domestic Appliances	Kitchen utensils, dishwashers, washing machines, vacuum cleaners.
Miscellaneous	Coins, keys, doorknobs, eyeglass frames, razors.

Experiments have been carried out to study nickel's absorption through the skin. The barrier to penetration is the stratum corneum. Percutaneous uptake appears to be mainly through sweat ducts and hair follicles (Wells, 1956). Absorption is probably slow. Using surgically removed skin, Fullerton et al. (1986) have suggested that nickel takes approximately 50 hours to permeate the epidermis and dermis of occluded skin and does not permeate non-occluded skin.

Occlusion of skin in vivo will produce friction, heat and sweat, all of which increase the penetration of nickel. Synthetic sweat solutions can leach nickel from various materials (Katz and Samitz, 1975) and temperature elevation increases nickel release from some coins and jeans buttons (Menne and Solgaard, 1979).

Sensitivity to nickel is commonly investigated by patch testing with 5% nickel sulphate in petrolatum, a concentration which rarely sensitises (Agrup, 1968), but which may be mildly irritant to some patients. Patch testing with 2.5% nickel sulphate may fail to detect 20% of sensitised patients (Cronin, 1980). A proportion of nickel-sensitive patients may have associated allergy to two other transition metals, cobalt and chromium. Approximately one third of nickel reactive women and one sixth of men also respond to cobalt. One tenth of nickel reactive men also react against chromium, but joint nickel-chromium reactivity in women is rare (1%), (Cronin, 1980). The three transition metals are not cross-reactive, but they occur together in several compounds and concomitant sensitisation may take place (Fregert, 1981).

In many countries nickel is the commonest sensitiser in the female (Cronin, 1980). Various studies have reported incidences of sensitivity between 8% and 15% in women and between 1% and 3% in men. In 1979 Peltonen undertook the first study of nickel sensitivity in the general population (ie not a population attending clinics). Nine hundred and eighty subjects above the age of 10 were patch tested with 5% nickel sulphate. Eight per cent of the females had positive patch tests and 0.8% of the males. It has been suggested that more females are allergic to nickel than males because of repeated exposure from jewellery, clothing and stainless steel kitchen equipment. Evaluation of 185 materials (using the maximisation procedure for identifying contact allergens) indicated that sex differences, in the prevalence of sensitisation, reflected previous exposure (Leyden and Kligman, 1977). During the last twenty years the age of the onset of nickel allergy in women has decreased from 30-50 years to 11-20 years (Cronin, 1980). A

relationship between nickel sensitivity and ear piercing (which has become popular in recent years, particularly in younger women) has been shown by Larsson-Stymne and Widstrom (1985), who studied 960 schoolgirls and found that the prevalence of nickel allergy among girls with pierced ears is 13% and in those without pierced ears 1%.

Different clinical forms of nickel dermatitis, have been described.

1) Localised dermatitis at an area in direct contact with nickel (see Table 1.5, figs 1.10 & 1.11).

Table 1.5 Sites and Sources of Nickel Dermatitis

Location	Nickel Source
Scalp	Hairpins, curlers.
Eyelids	Eyelash curlers.
Earlobes	Earrings.
Back of ears	Spectacle frames.
Lips	Metal pins held in mouth, metal lipstick case.
Neck	Clasp of necklace, zipper.
Upper chest	Medallions, buttons.
Axilla	Zipper.
Breast	Wire support of bra cup.
Abdomen	Buttons on jeans.
Palms	Handles of doors, handbags.
Fingers	Thimbles, needles, scissors, coins, pens.
Wrists	Watch bands, bracelets.
Arms	Bracelets.
Ankles	Bracelets.
Dorsum of foot	Metallic eyelets of shoes.
Postoperative site	Screws, bolts, plates in orthopaedic implants.

Modified from Fisher, 1975

Localised Dermatitis at Areas in Direct Contact with Nickel

Fig 1.10 Jewellery Dermatitis

Fig 1.11 Allergy to Nickel in Jean Studs

2) Secondary eruption of varying severity - initially considered to be unrelated to nickel contact but may result from inadvertent nickel contact (Fisher, 1975). Secondary spread is usually eczematous and often symmetrical; however non-eczematous reactions (eg urticaria or generalised itch) may occur.

3) Hand dermatitis - occurring, at some time, in approximately 40% of nickel-sensitive women (Menne et al., 1982). Eight to 11% of all cases of hand eczema are caused by nickel sensitivity; however, atopy, wet work and exposure to irritants and sensitisers may also predispose an individual to hand dermatitis (Rystedt, 1985; Fregert, 1975).

Hand eczema has a poor prognosis and presents in many patients as pompholyx (Christensen and Moller, 1975a) which is a recurrent vesicular eruption primarily located on the palms, and volar aspects and sides of the fingers. Christensen and Moller (1975b) suggested that pompholyx could be perpetuated by oral nickel ingestion after aggravating hand eczema with 5-6mg nickel. However, lower doses do not exacerbate pompholyx (Gawkrodger et al., 1986). The exact role of dietary nickel in aggravating nickel dermatitis has not been determined but a diet intended to reduce the daily intake of nickel may improve some cases of nickel dermatitis (Veien et al., 1985).

A low nickel diet is restrictive and is not suggested in the management of all cases of nickel dermatitis. Patients are, however, informed of the need for good skin care (particularly hand care) and instructed to reduce external nickel contact (eg varnishing jeans buttons or covering them with material).

Free (allergenic) nickel in metallic objects can be detected using the dimethylglyoxime (DMG) spot test :-

A few drops of 1% dimethylglyoxime in alcoholic solution plus a few drops of 10% ammonium hydroxide solution produce a strawberry red insoluble salt in the presence of available nickel (Fisher, 1975).

Sites of chronic dermatitis are commonly treated with topical corticosteroids to alleviate itching and inflammation.

METHODS FOR THE DETECTION OF NICKEL HYPERSENSITIVITY

In Vivo Detection

At present, a diagnosis of nickel sensitivity is based on patch testing with 5% nickel sulphate in petrolatum, often with a battery of other allergens, and this concomitant patch testing may lead to false positive results (Mitchell, 1977). In addition, patch testing may occasionally sensitise non-allergic individuals (Agrup, 1968) and should be carried out when the patient's back is free of eczema (Mitchell, 1975).

Difficulties may be encountered when interpreting patch test sites. Patch testing may cause irritant reactions (fig 1.12) and these may be mistaken for allergic reactions. Patients with atopic eczema may produce false positive pustular reactions to nickel sulphate patch tests (Fisher, 1975).

Finally, patch testing requires patient attendance at the hospital three times in one week, and can aggravate a patient's previous or existing dermatitis. The patch test reactions (with itch) may cause discomfort to the patient and occasionally patch testing may cause the 'angry back syndrome' (Mitchell, 1975) (fig 1.13).

Therefore an *in vitro* method for detecting nickel sensitivity would possess some advantages. However, it then becomes necessary to obtain blood samples from patients, which can be unpleasant for the patient and is also potentially hazardous to both clinicians and laboratory workers.

Fig 1.12 Difficulties Encountered when Interpreting Patch Tests

Patch test reactions:-

Top left = irritant Top Middle = +? Bottom Right = ++

Fig 1.13 Angry Back Syndrome

In Vitro Assays

Three assay methods have been described for detecting nickel sensitivity *in vitro*.

(1)Leucocyte Procoagulant Activity

The blood coagulation system is involved in the earlier stages of a delayed hypersensitivity reaction. Macrophages, immobilised by lymphokines (including migration inhibition factor (MIF)), are surrounded by fibrin, which induces macrophage production of plasminogen activator. In addition, activated T-cells secrete a lymphokine to induce monocyte procoagulant activity. An *in vitro* method to determine procoagulant activity was first described in 1982 (Geczy and Meyer) and has been used in preliminary experiments to identify nickel-sensitive patients (Aldridge *et al.*, 1985). Ten out of twelve nickel-sensitive patients could be discriminated from 9/9 controls. The two patients who did not respond had had quiescent disease for several months, but still gave positive patch test reactions. Therefore, detection of nickel sensitivity using this assay probably requires currently active disease.

(2)Macrophage Migration Inhibition Test

Migration inhibition factor (MIF) was the first lymphokine to be described, and can be detected 4-6 hours after lymphocyte activation *in vitro*. The macrophage migration inhibition test (MMIT) assays the release of antigen-induced MIF from activated T-cells. To detect nickel sensitivity, supernates from nickel-activated or non-stimulated T-cells are incubated with monocytes or macrophages, and the ability of the cells to migrate through agarose or from a capillary tube is measured.

Lymphokine release is thought to represent an important step in the development of delayed hypersensitivity reactions; however, results obtained in the MMIT using the antigen nickel sulphate have been inconsistent (Mirza *et al.*, 1975; Nordlind and Sandberg, 1983). Combining the MMIT with another *in vitro* assay, the

lymphocyte transformation test (LTT) may provide a reliable diagnosis in most patients tested for nickel allergy (von Blomberg-van der Flier et al., 1987).

(3)Lymphocyte Transformation Test

Studies on the in vitro activation of lymphocytes by specific antigens or non-specific mitogens (eg the lectins Concanavalin A (Con A) and phytohaemagglutinin (PHA)) with associated transformation into blast cells began in the late 1950s. It took another 15-20 years for the presently used lymphocyte transformation test (LTT) to be developed.

Method Development

Initial lymphocyte transformation studies were hampered by difficulties in obtaining leucocytes from whole blood. Preliminary techniques utilised the ability of PHA to agglutinate erythrocytes which could then be separated from leucocytes by centrifugation (Hungerford et al., 1959). However, PHA is a T-cell mitogen, therefore this method was useful only for investigating lymphocyte stimulation by PHA.

Lymphocyte enrichment was later obtained by methods involving gelatin (Coulson and Chalmers, 1964), passage through glass wool (Brandt et al., 1962) or glass beads (Schrek and Stefani, 1964). Also, a magnet was used to remove cells which had phagacytosed non-toxic carbonyl iron (Carstairs, 1962). The removal of the phagocytic cells prevented antigen-specific lymphocyte transformation (Oppenheim et al., 1966; cited by Pentycross, 1968) because of the now recognised requirement for accessory cells (which include monocytes).

At present, the most frequently used method for obtaining mononuclear cells is density sedimentation by centrifugation in Ficoll-sodium metrizoate (Ficoll-Paque) according to Boyum (1968); erythrocytes and neutrophils are pelleted, and the mononuclear cells are retained at the interface. Percoll (polyvinyl

pyrrolidone-coated silica in water) has also been used to isolate mononuclear cells. Nordlind (1984) stated that greater significant differences between nickel-sensitive patients and controls were obtained in a nickel sulphate LTT using Percoll instead of Ficoll-Paque. However, both patient and control cells were stimulated by the nickel sulphate at the concentrations studied. Prior to the use of Ficoll-Paque, separation methods may have damaged the leucocytes; cells were viable before culture, but after a 6-day culture, viability decreased to 44-53% (Hughes and Caspary, 1970).

Constituents of Tissue Culture Medium

Constituents of the medium (TCM) used in the LTT have been maintained during the development of the assay. However, the serum or plasma source (autologous or human AB serum) to be used remains a controversial issue. Autologous serum may contain antibodies capable of neutralising antigen, thus decreasing antigen availability for lymphocyte stimulation (Pentycross, 1968). In addition, certain substances (eg steroids) which inhibit lymphocyte stimulation (Casey and McCall, 1971) may be present in the serum of certain individuals, indicating that pooled human AB serum may be more suitable for use. However, factors in autologous serum may mimic the *in vivo* situation more accurately. Furthermore, the use of autologous plasma in the LTT may provide better discrimination between sensitised patients and negative controls (Hicks *et al.*, 1986). Therefore, autologous plasma was used in the assay system described in this thesis.

Measurement of Transformation

Lymphocyte transformation was initially quantified by the morphological assessment of blast cells in prepared smears. The percentage of cells scored as transformed depends on a number of variables, including the technique used for film preparation, the counting procedure and the definition of a transformed cell. Furthermore, substances initiating cell clumping (eg PHA) make many cells morphologically unrecognisable (Pentycross, 1968). Reliable results are obtained only by counting at least 4000 cells. After

cell transformation different cell morphologies exist including:-

(1) cells with obvious mitotic figures

(2) large (20-40μm) transformed cells with stippled nuclear chromatin, visible nucleoli and abundant basophilic cytoplasm

(3) small and medium lymphocytes

(4) dead cells

Approximately 90% of the cells belong to categories 2 and 3 (Pentycross, 1968).

It should be remembered that whilst the first morphological studies were being performed, little was known about the role of lymphocytes in the immune response. In 1962, Carstairs suggested that the blast cells may have originated from one of three sources-contaminating multipotential cells, medium and large lymphocytes, and the small lymphocytes (the source supported by his data).

Lymphocyte transformation studies employing a second technique (autoradiography) with tritiated thymidine and colchicine showed that the mitotic figures in PHA-enriched leucocytes present after culture were transformed lymphocytes and did not arise from cells in DNA synthesis at time zero, or from other small populations of cells (Mackinney et al., 1962). The authors concluded "...that the sequence of morphological patterns of mononuclear cells represented developmental stages or transformation of the small lymphocytes."

The technique now commonly used to study lymphocyte transformation is the estimation of radioactive thymidine uptake into cells by liquid scintillation counting (Caron et al., 1965). This technique provides a quantitative method of assessing lymphocyte transformation in vitro without requiring identification of particular cells. Initially, dissolution of the cells in hyamine was required; later, a less time-consuming millipore filtration technique was developed (Robbins et al., 1972). The advent of microculture in the mid 1970s and the use of automatic cell harvesters have increased the efficiency, rapidity and sensitivity of the assay.

The Lymphocyte Transformation Test In The Diagnosis Of Nickel Allergy

The LTT has become a valuable research technique and has been used widely to study the role of lymphocytes in nickel hypersensitivity.

Nature of Antigen Recognised

Three different nickel salts have been used in the LTT; nickel acetate, nickel chloride and nickel sulphate. However, the actual antigen which induces specific lymphocyte transformation is unknown. Nickel may conjugate with amino acids or proteins in serum, bind directly to lymphocytes or react with serum components to produce a sensitiser (Hutchinson et al., 1975). Using radioactive nickel ^{63}Ni, Hutchinson et al. (1975) showed that lysine was a major binding amino acid and that nickel also binds to approximately 20% of lymphocytes from both nickel-sensitive individuals and controls. Nickel-binding proteins in serum and peripheral blood mononuclear cells are not different in nickel-sensitive patients and controls (Silvennoinen-Kassinen et al., 1987). Veien et al. (1980) investigated nickel binding to cells and showed that 90-99% of the nickel could be removed in three thirty minute washes. However, nickel remaining on the pulsed cells could still initiate lymphocyte transformation. The authors also suggested that albumin-nickel (described by Lucassen and Sarkar, 1979) was not bound to the lymphocytes. Direct binding to lymphocyte membranes was postulated by Nordlind and Henze (1984) when they studied the effects of nickel sulphate on thymocytes in the presence or absence of serum. Earlier investigators had concluded "One can do no more than speculate on the nature of the antigen in our in vitro experiments for the medium, consisting of plasma, medium 199, and disintegrating cells, offers a multitude of amino acid complexes, peptides, and proteins, to the nickel hapten." (Macleod et al., 1970).

The first investigators of nickel-specific lymphocyte transformation used morphological criteria to establish specific stimulation by nickel acetate and described transformation in cells from both nickel-sensitive individuals and controls (Aspegren and Rorsman, 1962). These observations were supported by Pappas et al. (1970) who incubated nickel acetate (10-30µg/ml) with cells from eight controls and three nickel-sensitive individuals, and carried out morphological and autoradiographic studies. The same response was evident in all eleven cultures.

Using liquid scintillation counting and the antigen nickel chloride (at concentrations of 2.5-30µg/ml), Millikan et al. (1973) described nickel-specific lymphocyte transformation (in contrast to a non-specific mitogenic response). However, the antigen used in LTTs to detect nickel sensitivity is commonly nickel sulphate. Variable results obtained from some previous LTTs using nickel sulphate to detect nickel sensitivity are shown in Table 1.6.

In the first study carried out by Macleod et al. (1970), only one nickel sulphate concentration (14µg/ml) was studied and 7/12 of the nickel-sensitive patients were positive in the LTT. Cells from the controls were not stimulated, suggesting that 14µg/ml nickel sulphate was not mitogenic. Forman and Alexander (1972) also claimed that the LTT using nickel sulphate (concentrations not stated) would provide a useful in vitro test for detecting nickel-sensitive individuals.

Gimenez-Camarasa et al. (1975) used serial dilutions of nickel sulphate in the LTT to detect nickel sensitivity and described two types of specific response. Fourteen patients had maximum thymidine incorporation at the lowest nickel sulphate concentration (0.1µg/ml); conversely, eleven patients showed maximum thymidine incorporation at the highest salt concentration (10µg/ml). Svejgaard et al. (1978) found that 3/16 controls reacted

Table 1.6 Summary of Results from LTTs Using Nickel Sulphate To Detect Nickel Sensitivity

Authors	Assay (days)	SI	Nickel Sulphate concentrations (µg/ml)	Detected as positive			
				Nickel Sensitive		Controls	
				No.	%	No.	%
Macleod et al. (1970)	6	2	14	7/12	58	0/14	0
Forman and Alexander (1972)	4	2	?	15/18	83	0/10	0
Hutchinson et al. (1972)	6	2	14	6/8	75	0/7	0
Gimenez-Camarasa et al. (1975)	6	2	0.1-10	23/25	92	0/10	0
Svejgaard et al. (1978)	5	3	0.9-19	7/8	88	2/16	13
Al-Tawil et al. (1981)	5+6	Variable	6.25-25	15/16	94	0/10	0
Macleod et al. (1982)	6	2	14	4/6	67	2/6	33
von Blomberg-van der Flier et al. (1987)	6	2	2-20	25/27	93	9/23	39
Res et al. (1987)	5	2	?	11/13	85	Not tested	

significantly in the LTT and they suggested that nickel sulphate may be mitogenic or that the LTT is more sensitive than patch testing to identify nickel-sensitive individuals.

It is therefore necessary for investigators using the LTT to define carefully parameters of the assay which distinguish nickel-sensitive patients from negative controls. In 1981, Al-Tawil et al. outlined criteria related to stimulation indices of patients and controls on days 5 and 6 of culture using 6.25-25 µg/ml nickel sulphate. Although they were successful in detecting 15/16 patients, the criteria they employed were complicated (requiring two different stimulation indices, three different nickel sulphate concentrations and comparing the results with non-sensitised controls). Therefore, the description of an assay system which can discriminate easily between nickel-sensitive patients and controls would be valuable and would remove the necessity for patch testing.

OUTLINE OF THE INVESTIGATION

The overall aim of the work described in this thesis was to use and adapt the LTT to investigate the *in vitro* presentation of nickel sulphate to cells obtained from nickel-sensitive patients and negative controls. Two aspects of nickel presentation were studied:-

(1) The abilities of blood and epidermal cells to present nickel to enriched T-cells (Chapter 4).

(2) The role of MHC II antigens (HLA-DP -DQ -DR) in nickel-specific T-cell proliferation (Chapter 5).

However, it was first necessary to establish a LTT which could discriminate between nickel-sensitive patients and non-sensitised controls (Chapter 3).

MATERIALS AND METHODS

PATCH TESTING

Patch testing equipment is shown in fig 2.1. Commercially prepared allergen (Trolab, Denmark) was placed in an aluminium Finn chamber (Pirila, 1975) and applied to the patient's upper back, using Scanpor (Epitest Ltd, Finland) avoiding the skin over the vertebral column (fig 2.2). This site produces the strongest allergic and irritant reactions (Magnusson and Hersle, 1965). The Finn chamber's design (diameter 8mm, depth 0.5mm) promotes good occlusion, localises reactions to the test sites and permits the use of porous tape for patch test application.

Test sites were marked with pen on the patient's back and the chambers removed after 48 hours. Patients were instructed to protect the test sites from water and to avoid scratching reacting sites. Sites were examined half an hour after patch test removal, allowing time for a positive reaction to become evident. Patients returned for a second reading at 120 hours when most allergic reactions would be present, but irritant reactions would have subsided.

Patch test results were scored using a recognised recording system recommended by the ICDRG (Fregert and Bandmann, 1975):

Table 2.1 Scoring of Patch Test Results

-	Negative
+?	Doubtful reaction; faint erythema
+	Weak; palpable erythema, infiltration, possibly papules.
++	Strong; erythema, infiltration, papules, vesicles
+++	Extreme; intense erythema and infiltration, coalescing vesicles

Patch test reactions are shown in figs 2.3 and 2.4.

Fig 2.1 Patch Testing Equipment

Commercially prepared antigen (in the syringe) is placed in the aluminium chambers.

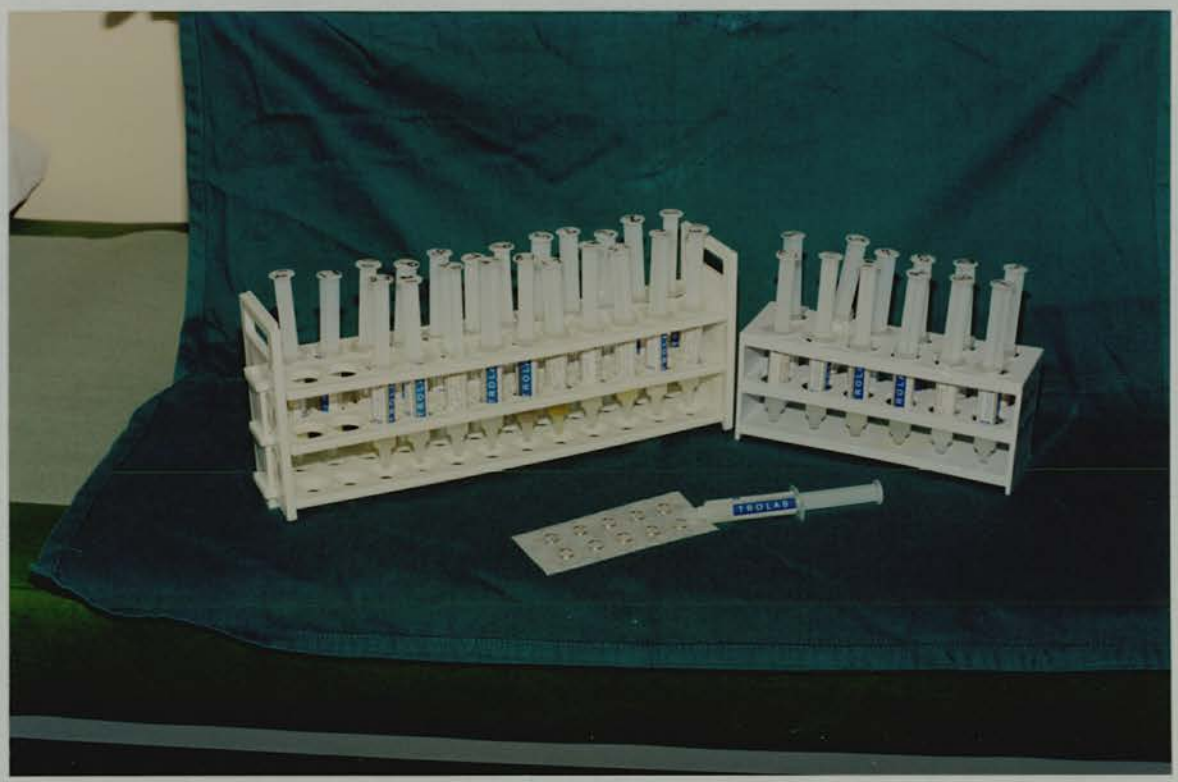
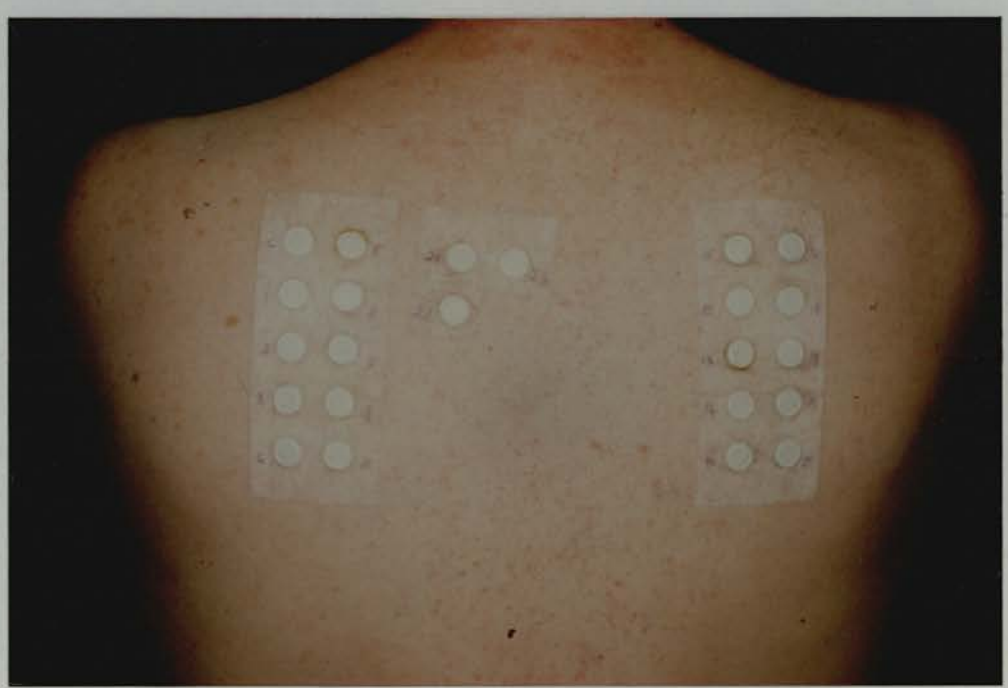


Fig 2.2

The chambers are then applied to the patient's upper back.



Patch Test Reactions
Fig 2.3 Top left = + reaction Bottom right = +++ reaction

Fig 2.4 +++ reaction against $NiSO_4$ (Patient 27)
The reaction follows the path of the draining lymph node.

PATIENTS AND CONTROLS

Patients

Patients in this study are defined as those subjects with positive patch tests to 5% nickel sulphate when tested with the European Standard Battery of contact allergens (Table 1.3, page 22). Blood samples were obtained from most patients when they attended the patch test clinic for the 120hr reading.

Peripheral blood mononuclear cells from two patients, Pa and Pb (nickel patch test reactions +++(48hr) ++(120hr) and ++(48hr) ++(120hr) respectively) were used to establish the toxicity of nickel sulphate in a 5 day LTT.

A review of patients studied in the 6 and 7 day LTTs with the results of patch testing and clinical manifestations is shown in Table 2.2.

Key For Tables 2.2 and 2.3

Cr	= Chromium	Form	= Formaldehyde
BoP	= Balsam of Peru	Frag	= Fragrance mix
Co	= Cobalt	Col	= Colophony
Neo	= Neomycin	Prim	= Primin
Cai	= Caine mix	W.T.	= Wool Tars
N.A.	= Not Available (but known to be positive)	N.T.	= Not Tested

Controls

Controls in this study were mostly healthy volunteers who had no history of metal contact dermatitis (C7-C46); however, some controls were patients attending the patch test clinic with histories suggesting nickel sensitivity but who did not react to the nickel sulphate patch test (C1-C6). Information about the controls is shown in Table 2.3.

Table 2.2 Details of Patients Studied

No.	Sex (M/F)	Age	P.T. Date	Nickel P.T. Reactions 48hr	Nickel P.T. Reactions 120hr	Other P.T.	Other P.T. Reactions 48hr	Other P.T. Reactions 120hr	Clinical Manifestations And Other Information
P1	F	45	1/86	++	++				Jewellery reaction
P2	F	60	1/82	++	+++				Hand dermatitis
P3	F	35	3/86	+++	+++	Co	-	+	Jewellery reaction
P4	F	30	5/85	++	++	Co	+	+	
P5	F	47	8/82	+++	+++				Hand dermatitis
P6	F	66	4/86	++	++	BoP	+	+	Jewellery reaction
P7	F	30	7/86	++	++	Cr	+	+	Hand and wrist eczema
						Frag	+	+	
P8	F	61	5/86	-	+	Co	-	+	Reacts to watch
P9	F	26		++	++	Co	-	+	Hand dermatitis
P10	F	28	8/86	+?	+	Form	+?	+	Atopic eczema
P11	F	19	8/86	++	+++	Co	+	+	Blisters on palms
P12	F	26	8/86	+	+	Co	++	++	Microvesicular on palms
						Cr	-	++	
P13	F	22	9/86	+	++	Co	-	+	Probably atopic
P14	M	59	6/83	+++	++	Co	++	++	Pompholyx
						Cai	+++	++	
						W.T.	++	+	
P15	F	32	10/86	N.A.	N.A.				Hand dermatitis
P16	F	56	10/86	++	++				Chronic palmar eczema
P17	F	56	10/86	++	++				
P18	F	33	11/84	++	++	Co	+	+	Jewellery reaction
						Frag	+	+	
P19	F	26	11/86	++	++	Co	+	+	Recurrent eczema
P20	M	22	11/86	++	++				Wrists vesicular
P21	F	36	4/86	+	+	Co	-	+	Atopic. Wet work
						Frag	+	+	
P22	F	22	12/86	++	++	Co	++	++	Constitutional eczema
						Frag	++	++	
P23	F	61	12/86	+	++				Hand dermatitis

Table 2.2contd

No.	Sex (M/F)	Age	P.T. Date	Nickel P.T. Reactions 48hr	Nickel P.T. Reactions 120hr	Other P.T.	Other P.T. Reactions 48hr	Other P.T. Reactions 120hr	Clinical Manifestations And Other Information
P24	F	38	1/87	-	++				Foot dermatitis
P25	F	22	1/87	++	++	Form	+	+	Jewellery reaction
						Col	+	+	
P26	F	16	1/87	++	++	Col	-	+	Earring reaction
P27	F	26	2/87	+++	+++				Jewellery reaction
P28	M	22	2/87	++	++	Dyes	+	+	Mild hand dermatitis
P29	F	25	7/83	+++	+++	Form	-	+	Blisters within minutes of contact. Angry back
P30	F	70	2/87	+	++	Cr	-	+	Hyperkeratotic
						Co	++	++	dermatitis
						Frag	+	++	Slight earring reaction
						Prim	-	++	
P31	F	24	2/87	+?	++	Co	-	+	
P32	M	43	2/87	+?	+	Cr	++	+++	Foot eczema
						Co	+	++	
						Form	+	+	
P33	F	22	3/87	+?	+				Hand eczema. Asthma
P34	F	42	2/87	-	+				Erythrodermic dermatitis
P35	F	16	4/87	+	+				Atopic eczema. Hand eczema
P36	F	17	3/87	-	+				Hand dermatitis
P37	F	23	3/87	++	++	Co	+	+	
						Form	++	++	
						Para	+	++	
P38	F			+	++				
P39	F	30	4/83	++	++				Constitutional pompholyx
P40	M	38	1/87	++	++	Cr	++	++	Cement worker
						Co	+	+	
P41	F	56	2/86	+++	+++				Jewellery reaction
P42	F	45	4/87	+	++				Works with metal pins

Table 2.2 contd

No.	Sex (M/F)	Age	P.T. Date	Nickel P.T. Reactions 48hr	Nickel P.T. Reactions 120hr	Other P.T. Reactions	Other P.T. Reactions 48hr	Other P.T. Reactions 120hr	Clinical Manifestations And Other Information
P43	F	18	4/87	+	++	Form	+	++	Atopic eczema
P44	M	46	4/87	+	++				Microvesicular palms
P45	F	20	3/87	+	++	Form	+++	+++	Earring reaction
P46	F	19	6/87	+	++	Neo	+	++	Earring reaction
P47	F	16	6/87	+++	++				Hand dermatitis
P48	M	56	5/87	+++	+++				Wrist watch reaction
P49	F	33	6/87	N.A.	++				
P50	F	26	6/87	+	++	Cr	-	+?	Jewellery reaction
P51	F	63	7/84	+++	+++	Co	++	++	Jewellery reaction
						Frag	++	++	Works with metal cards
						Neo	-	++	
P52	F	18	6/87	+++	+++				Vesicular hand eczema
P53	F	75	7/87	+	++				Jewellery reaction
P54	F	17	7/87	++	++				
P55	M	29	8/87	+	++	Cr	+	+	Hand dermatitis
						Col	+	+	
P56	F	27	8/87	+?	+	Form	++	++	Cheiropompholyx
P57	F	17	8/87						
P58	F	20	8/87	+	+	Cr	+	+	
						Co	+?	+	
P59	F	32	8/87	-	+				Mild eczema
P60	F	19	8/87	+	+				Jewellery reaction
P61	F	18	8/87	N.A.	N.A.				
P62	F	21	8/87	+	++	Co	+	++	Earring reaction
P63	F	17	10/87	++	++	Col	+++	+++	Jewellery reaction
						Frag	++	++	
						Form	++	++	
						BoP	+	+	
P64	F	32	10/87	+	+	Frag	++	++	Wrist dermatitis
						Col	-	++	
P65	F	18	11/87	+	+	Not tested			
P66	F	40	11/87	+++	+++				Jewellery reaction

Table 2.3 Details of Controls Studied

(a) Patch Test Negative Patients

No.	Sex (M/F)	Age	P.T. Date	Nickel P.T. Reactions 48hr	Nickel P.T. Reactions 120hr	Other P.T. Reactions	Other P.T. Reactions 48hr	Other P.T. Reactions 120hr	Clinical Manifestations And Other Information
C1	F	34	1/87	-	-				Hand eczema. Atopic eczema previously
C2	F	43	2/87	-	-	Col	-	+	Hairdresser. Eczema of fingertips
C3	F	47	1/87	-	+?	Prim	+	+	Irritant eczema
C4	F	27	3/87	-	-				
C5	M	39	3/87	-	-				Probably psoriasis
C6	F	17	3/87	-	-				

(b) Controls With No History of Metal Contact Dermatitis

No.	Sex (M/F)	Age	No.	Sex (M/F)	Age	No.	Sex (M/F)	Age
C7	F	23	C21	M	31	C34	F	50
C8	F	50	C22	F	38	C35	F	45
C9	F	35	C23	M	33	C36	F	23
C10	F	24	C24	F	25	C37	F	54
C11	F	54	C25	M	30	C38	F	51
C12	F	29	C26	M	20	C39	F	25
C13	F	23	C27	M	32	C40	F	22
C14	M	34	C28	F	22	C41	F	35
C15	F	24	C29	F	54	C42	F	49
C16	F	34	C30	M	30	C43	F	34
C17	F	29	C31	M	49	C44	F	51
C18	F	43	C32	M	31	C45	F	30
C19	M	25	C33	M	31	C46	F	51
C20	M	33						

MEDIUM AND BUFFERS

Tissue Culture Medium

RPMI 1640
100IU/ml penicillin
100µg/ml streptomycin
0.002M L-glutamine
0.08% sodium bicarbonate (above purchased from Gibco, Paisley)
$2x10^{-5}$M 2-mercaptoethanol (Sigma, Poole)

Phosphate Buffered Saline (PBS)

Sodium chloride 8g
Disodium phosphate 0.2g
Potassium chloride 0.2g
Potassium dihydrogen orthophosphate 0.2g
Made up to 1 litre with distilled water

ELISA Substrate Buffer

0.1M glycine buffer
containing
0.001M magnesium chloride
0.001M zinc chloride

NAMES AND ADDRESSES OF SUPPLIERS

Materials used during the experiments are listed with the names and addresses of the suppliers in Appendix II (pages 175-77).

PREPARATION OF CELL POPULATIONS

Preparation of Peripheral Blood Mononuclear Cells

Blood (30-60ml) was obtained by venepuncture and placed in heparinised tubes (10IU heparin/ml, Sigma, Poole). Five ml blood was layered onto an equal volume of lymphocyte separation medium (Flow, Rickmansworth) in 10ml conical tubes and centrifuged (400g, 20 mins, room temperature). The plasma was collected, then the mononuclear cells (PBM) were harvested from the interface and washed three times in phosphate buffered saline (PBS).

Antigen Presenting Cells

The abilities of
(a) plastic-adherent blood cells and
(b) epidermal cells
to present Con A and $NiSO_4$ to enriched T-cells in the LTT were studied.

In these experiments, plasma was filtered to remove any contaminating cells and then added to TCM at a concentration of 15%. Once obtained, PBM and antigen presenting cells were maintained at $4^{o}C$ to prevent cell adherence.

(a) Plastic-Adherent Blood Cells

PBM were resuspended in TCM+15% autologous plasma, and incubated in plastic petri dishes at $37^{o}C$ for 45 minutes. The non-adherent cells were removed by washing the dishes with PBS. The plastic-adherent cells were removed from the dishes by gentle scraping with a 1ml syringe plunger. The cells were washed, resuspended in TCM+15% autologous plasma and irradiated with 3000 rads using ^{137}Cs (RX2/50 irradiator (Gravatom Industries Ltd)).

(b) Epidermal Cells

A method to obtain epidermal cells from suction blisters was originally described by Kistaala (1968). This method was adapted to obtain epidermal cells from patients and controls. Sterile suction devices with five holes of 5mm diameter were placed on the volar aspects of the upper arms and held in position by tape. A vacuum (-300mm Hg) was then applied to the devices for approximately 1½ hours until five blisters had been raised and were easily visible through the cups (fig 2.5). Blister roofs (fig 2.6) were removed aseptically and placed in PBS containing 500IU/ml penicillin, 500µg/ml streptomycin, 250µg/ml gentamycin and 12.5µg/ml fungizone for 45 minutes. Blister roofs were then incubated for 45 minutes with 0.3% trypsin at 37°C. The roofs were gently agitated to release epidermal cells and washed three times in TCM+5% foetal calf serum (FCS) to inactivate the trypsin. The stratum corneum was not pelleted and was easily removed with the washing solution. Cells were resuspended in TCM+15% autologous plasma and irradiated (3000 rads).

T-cell Enrichment

Enriched T-cells were prepared from the plastic-non-adherent blood cells by either

(a) T-cell rosetting or

(b) nylon wool passage

(a) T-Cell Rosetting

(1) Sheep Red Blood Cells (SRBC)

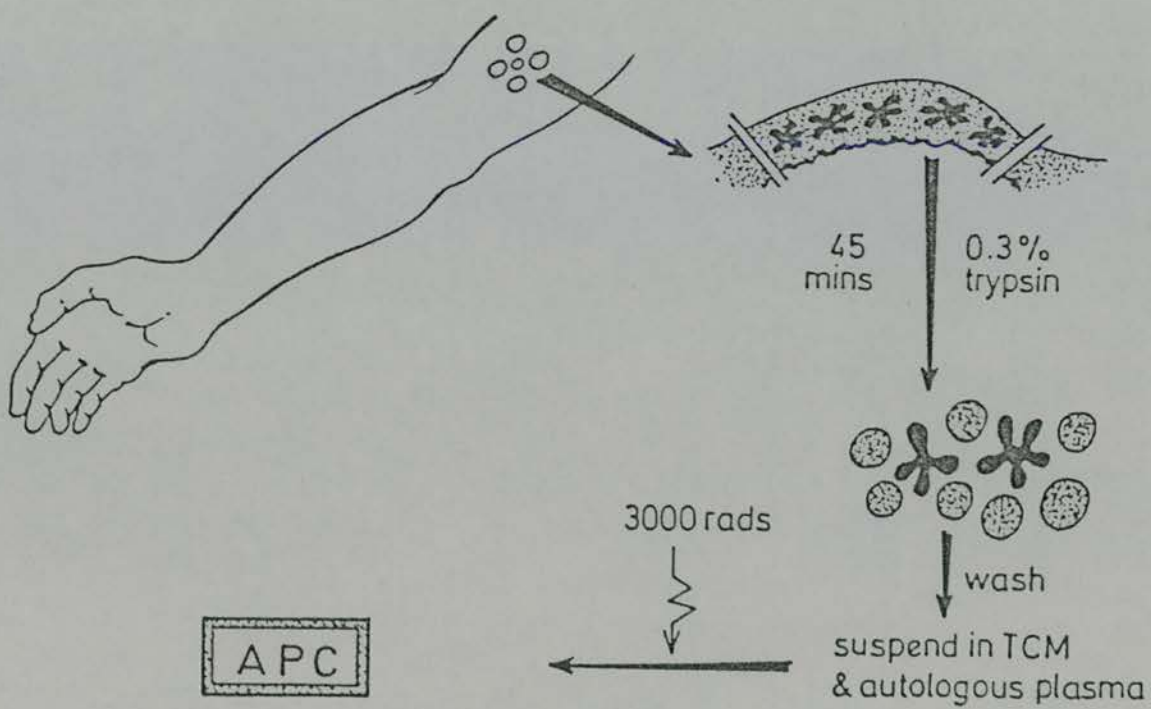
SRBC in 50% Alsever's solution (Moredun Research Institute, Edinburgh) were washed twice in PBS.

(2) AET-SRBC

One volume of packed SRBC was incubated with 4 volumes of aminoethylisothiuronium bromide hydrobromide (AET, Sigma, Poole) (4%w/v in saline, pH 9.0) for 15 minutes at 37°C. Cells were washed three times and resuspended at 2%v/v in TCM + 20% FCS.

Fig 2.5 Suction Blisters Raised on the Upper Arms

Fig 2.6 Preparation of Epidermal Accessory Cells



(3) Rosetting

Equal volumes of 2% AET-SRBC

FCS

and plastic-non-adherent blood cells ($3-4x10^6$/ml)

were mixed and pelleted (150g, 5mins, 4^oC)in a 20ml conical tube. Cells were incubated on ice for 90 minutes then the supernatant was removed. Rosettes were gently resuspended in TCM+30% FCS and clumps (which may contain non-rosetted cells) were allowed to settle at the bottom of the tube. The rosettes were layered onto lymphocyte separation medium (4^oC) and centrifuged (400g, 20mins, 4^oC). Cells at the interface were harvested, washed and resuspended in 0.5ml TCM+15% autologous plasma. The cells were mixed to dissociate the rosettes, then resuspended in 1ml lysis medium (1vol TCM + 5 vols distilled water) to remove the SRBC. Cells were mixed vigorously for 2 seconds, then 20ml TCM was added. Cells were washed in PBS, then resuspended in TCM+15% autologous plasma.

(b) Nylon Wool Passage

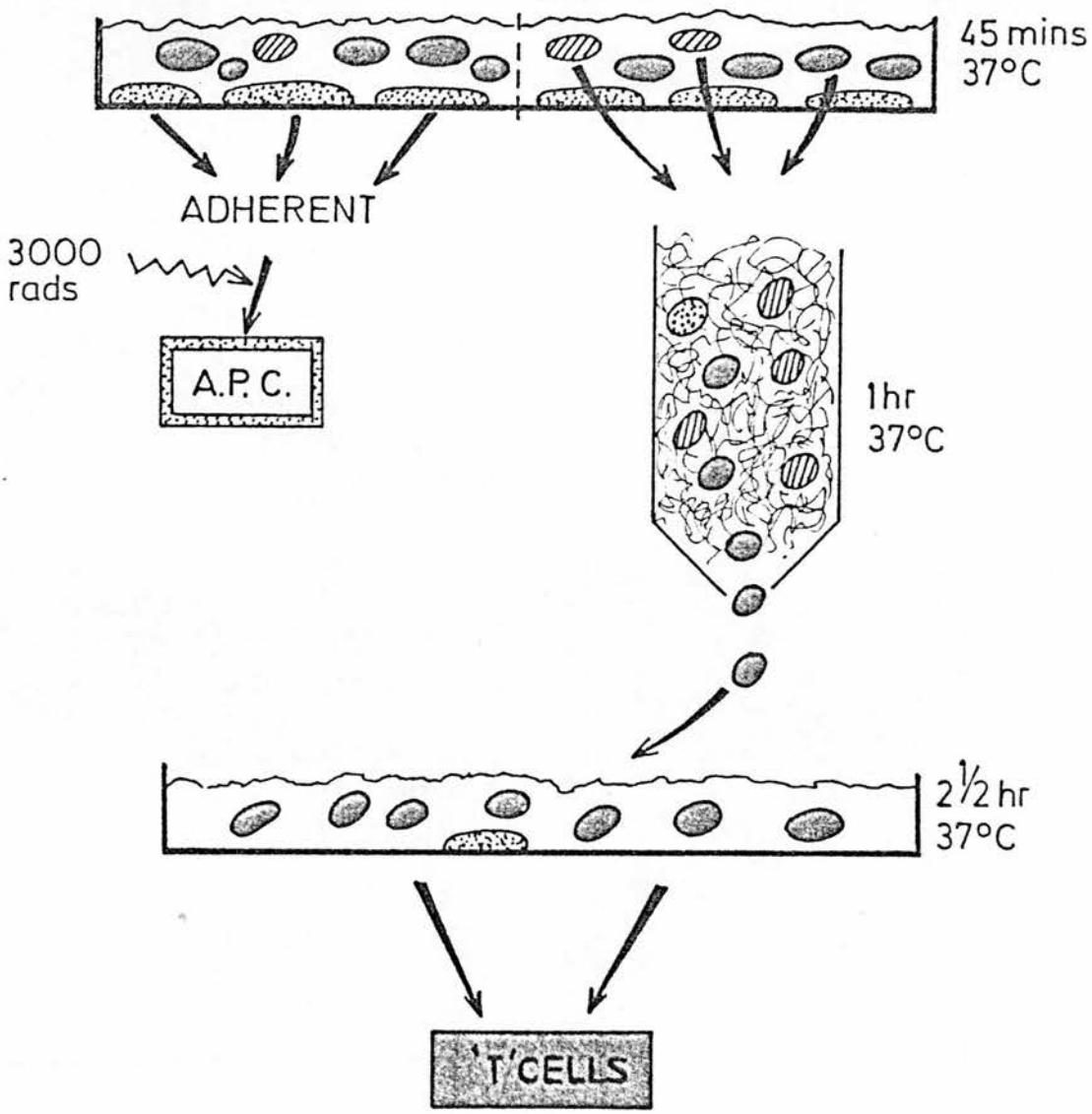
(1) Preparation of Nylon Wool Column

One g nylon wool (Fenwall, Illinois, USA) was packed into a 5ml syringe and sterilised. Columns were incubated at 37^oC with TCM+5% FCS for 1 hour before the addition of plastic-non-adherent cells.

(2) T-Cell Enrichment (fig 2.7)

Plastic-non-adherent blood cells were resuspended in approximately 0.7ml TCM+5% autologous plasma and incubated in the column for 1 hour at 37^oC. The cells were then washed from the column under the force of gravity using TCM+5% autologous plasma. Nylon wool-non-adherent cells were further incubated on glass for 2½ hours at 37^oC in TCM+15% autologous plasma. The glass-non-adherent cells were an enriched population of T-cells.

Fig 2.7 Summary of Method used for T-Cell Enrichment



Characterisation of Cell Populations

Whenever possible, the PBM, plastic-adherent blood cells, T-cells and epidermal cells were characterised by

a) The expression of HLA-DP-DQ-DR (using indirect immunofluorescence)

b) Staining of dendritic cells

Specific staining of dendritic cells using the monoclonal antibody RFD1 (Poulter et al., 1984) was unsuccessful at dilutions ranging between 1/5 and 1/100. The low numbers of dendritic cells in PBM made it impossible to ascertain the percentage of these cells present in the accessory cell population using two antibodies DA6.231 and Leu M3 (an antibody against monocytes/macrophages, Becton Dickinson, Teddington) (3% difference found).

Indirect Immunofluorescence

Cells ($2x10^5$) were incubated with 20% normal rabbit serum (NRS) in PBS for 10 minutes and then washed. Monoclonal antibody DA6.231 (anti HLA-DP-DQ-DR; Guy et al., 1982) was added at a dilution of 1/40 in 20% NRS for 40 minutes at 4^oC. Cells were washed three times in PBS, then 1/40 fluorescein conjugate (FITC-rabbit anti-mouse immunoglobulin (Fab fragment), Cappel, Philadelphia, USA)) was added for a further 40 minutes (4^oC). Cells were washed three times, mounted in glycerol and studied using a Leitz Ortholux II fluorescence microscope.

LYMPHOCYTE TRANSFORMATION TEST

Stimulants

Concanavalin A (Con A; Sigma, Poole) was used at a final concentration of 10µg/ml.

Nickel Sulphate ($NiSO_4.7H_2O$, Analar, BDH, Glasgow). A 1%w/v $NiSO_4.7H_2O$ stock solution was prepared in physiological saline, filter sterilised and stored at 4^oC. When required, the stock solution was diluted 1/20 in RPMI 1640 and added to the cells at final concentrations varying between 0 and 50µg/ml.

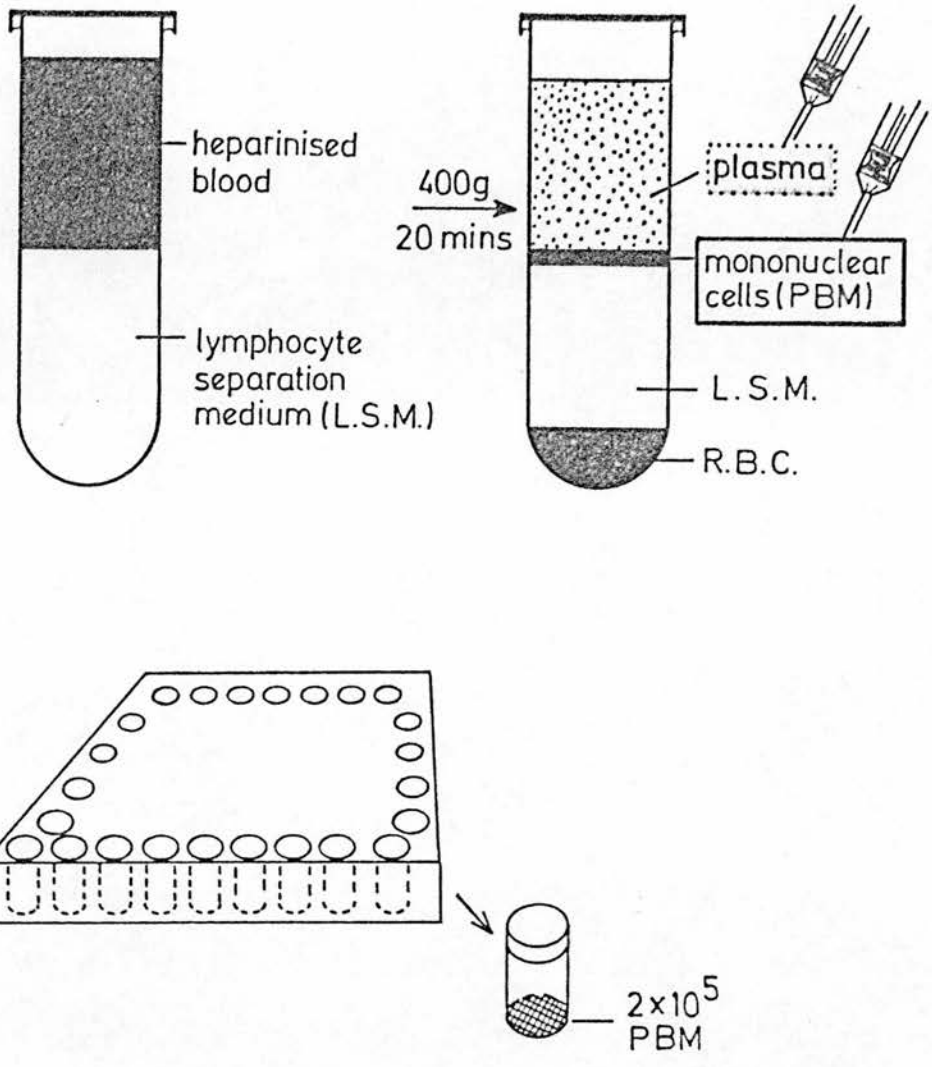
Peripheral Blood Mononuclear Cells (PBM) (fig 2.8)

Mononuclear cells were resuspended in tissue culture medium (TCM) containing 15% autologous plasma. An aliquot of cell suspension was diluted in trypan blue (Flow, Rickmansworth, final concentration 0.05%) to determine cell viability. Cells were counted using a Neubauer haemocytometer; viable cells, which exclude trypan blue, were expressed as a percentage of the total number of cells present.

PBM were diluted to a concentration of 10^6/ml in TCM+15% autologous plasma, then aliquoted into sterile 5ml culture tubes (Elkay, Basingstoke). Stimulant was added to the tubes and the solutions mixed. Aliquots (200µl) were placed in individual wells of a Linbro tissue culture plate. Cells ($2x10^5$/well) were then incubated for 6 or 7 days in a humidified Vindon incubator with 5% CO_2.

Lymphocyte transformation was measured by incorporation of ^3H-thymidine (Amersham); 1µCi of radiolabel was added to each well, 18-20 hours before harvesting cells onto filter discs (Skatron, Northumbria Biologicals, Cramlington) using a Dynatech automatic MASH II cell harvester. Filter discs were then placed in scintillation vials with 3ml scintillation fluid (2.51 toluene,

Fig 2.8 Summary of Method Using PBM in the LTT



12.5g 2,5-diphenyloxazole (PPO), 0.5g 1,4-di(2-methylstyryl)-benzene (Bis-MSB)); their radioactivity was determined using a Packard Tricarb 300 liquid scintillation counter.

Results obtained were expressed as a stimulation index (SI).

$$SI = \frac{\text{cells + stimulant (cpm)}}{\text{cells alone (cpm)}}$$

Stimulation indices greater than or equal to 3 were considered to be a positive response in the test (see results section).

Antigen Presentation By Plastic-Adherent Cells and Epidermal Cells

The LTT was carried out using the cell concentrations shown in Table 2.4.:-

Table 2.4 Cell Concentrations Used in LTT To Study APCs

Cell Types	Cell No/ml	%APC
PBM	10^6	
Enriched T-cells	10^6	
Plastic-adherent cells	10^5	
T + Adherent cells	$10^6 + 10^5$	10
T + Epidermal cells	$10^6 + 10^5$-$4x10^5$	10-40

Stimulant was added to the cells, the solutions mixed and 200µl aliquoted into individual wells. Plates were incubated for 6 days at 37°C with 5% CO_2 before cell harvesting.

Inhibition of LTT by Monoclonal Antibodies Against MHC II Molecules

The monoclonal antibodies studied in these experiments are shown in Table 2.5.

Table 2.5 Description of Monoclonal Antibodies Studied

Monoclonal Antibody	supernate/ ascites	Class	Supplier	Class II MHC recognised
DA6.231	ascites	IgG1	K. Guy	HLA-DP-DQ-DR
L243	supernate	IgG2a	S. Howie	HLA-DR
B7/21	ascites	IgG1	Becton Dickinson	HLA-DP
Leu-10	ascites	IgG1	Becton Dickinson	HLA-DQ
L368	ascites	IgG1	Becton Dickinson	$Beta_2$ micro-globulin

Becton Dickinson antibodies were dialysed against PBS for 48 hours to remove sodium azide preservative and then sterile filtered. Concentrations of the five antibodies studied were determined by ELISA (see page 63).

(1) Destruction of MHC II^+ Cells Using Antibody and Complement

PBM were incubated for 30 minutes at room temperature firstly with DA6.231 (1/5000 dilution), followed by rabbit anti mouse serum (1/100 dilution) and washed three times with PBS. The cells were warmed to 37°C and then guinea pig complement (Bacteriology Department, University of Edinburgh) (previously absorbed with SRBC and human PBM) was added (1/20 dilution). Cells were incubated with complement for 45 minutes at 37°C, then washed three times with PBS. Cells were resuspended at 10^6/ml in TCM+15% autologous plasma. Stimulant was added, the cells were placed in tissue culture plates and the LTT was carried out.

(2) Pulsing with Antibodies

After incubation with antibody for 1 hour at room temperature, 10^6 PBM were washed with PBS and resuspended in 1ml of TCM+15% autologous plasma. Stimulants were added to the cells and 200µl placed in the wells of a tissue culture plate. The LTT was then carried out.

(3) Maintaining Antibody in the Culture

Stimulants were added to PBM after they had been incubated with the antibodies for 1 hour at room temperature. The cells were then placed in tissue culture plates and the LTT carried out. In reconstitution experiments, antibody was incubated with the plastic-adherent blood cells for 1 hour before the addition of T-cells and stimulants.

ELISA TO DETERMINE MONOCLONAL ANTIBODY CONCENTRATIONS

Washings were carried out three times using PBS + 0.05% Tween

(1) Plate Coating

Anti mouse IgG (61µg/ml) (Sigma, Poole) was prepared in PBS in a glass container, and 100µl were added to the wells of an ELISA plate. Plates were incubated at room temperature for 1 hour then washed.

(2) Antibodies

Standard Curve

Mouse IgG (Sigma) was prepared at concentrations between 0-2000ng/ml in TCM+5% FCS.

Monoclonal antibodies were diluted in TCM+5% FCS. One hundred microlitres were added to triplicate wells and plates were incubated at room temperature for 90 minutes, then washed.

(3) Conjugate

Anti mouse IgG-alkaline phosphatase conjugate (Sigma) was diluted 1/400 in PBS and centrifuged (3000rpm, 20 mins) and 100µl were added to each well. Plates were incubated overnight at 4°C, then washed.

(4) Substrate

Para-nitrophenyl phosphate (1mg/ml) (Sigma) was prepared in substrate buffer (pH 10.4, see page 51). Colour was allowed to develop for 15 minutes at room temperature. The reaction was stopped by the addition of 25µl 3M sodium hydroxide. Plates were read using a Titertek MR580 Microelisa autoreader at 405nm.

RESULTS

DEVELOPMENT OF THE LYMPHOCYTE TRANSFORMATION TEST

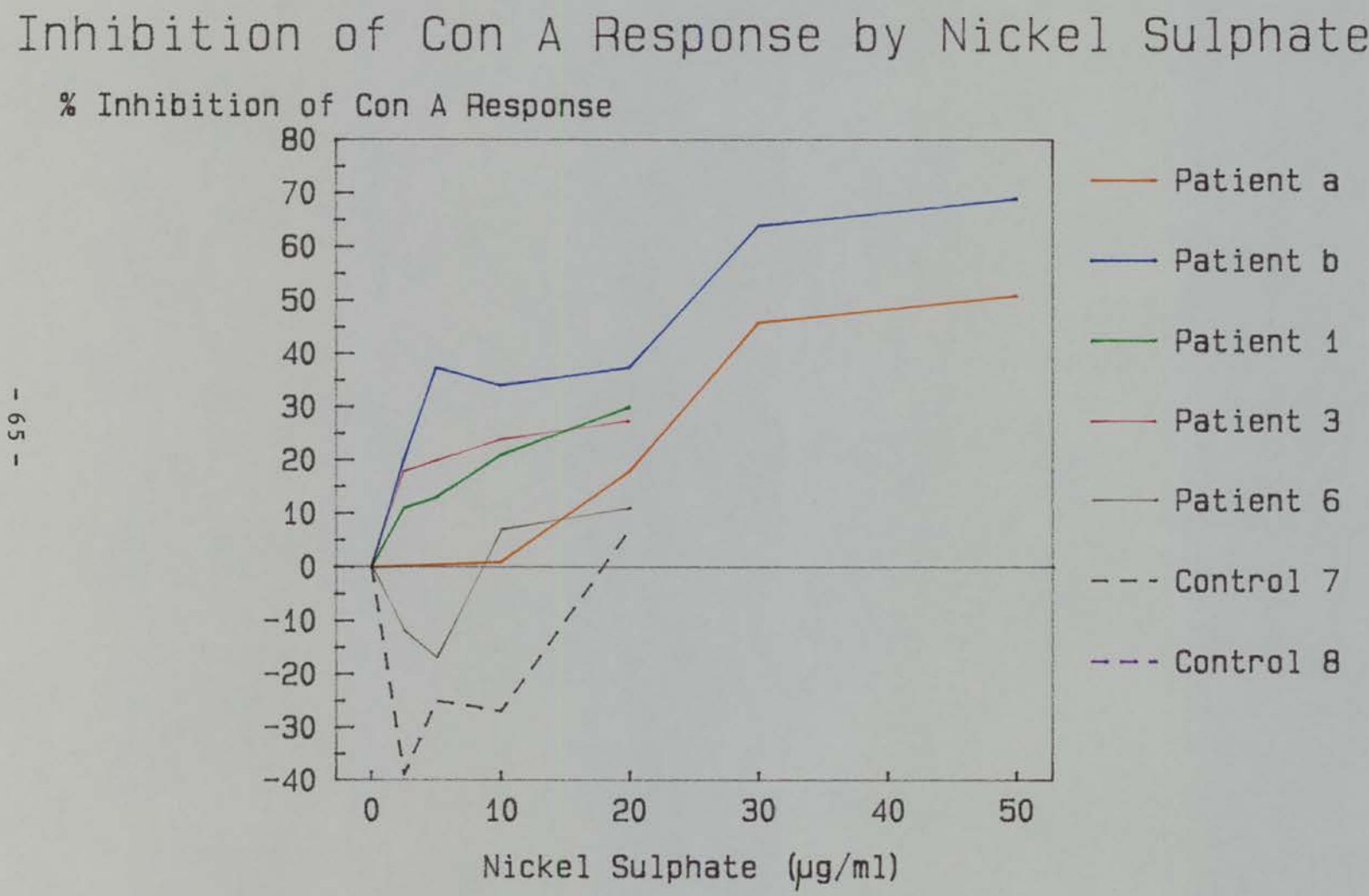
Before using the LTT to detect nickel sensitivity, it was necessary to establish optimal antigen concentrations and incubation periods for the assay. The method is described in detail on pages 58-60.

(1) Toxicity of Nickel Sulphate

Cells were incubated for 5 days with concentrations of nickel sulphate ranging from 0-50µg/ml in the presence of the mitogen Con A at a concentration of 10µg/ml.

Figure 3.1 shows the percentage inhibition of the response of cells to Con A by co-culture with increasing concentrations of nickel sulphate. Responses to Con A were similar in cells from patch test positive patients and controls. The Con A response was markedly inhibited at nickel sulphate concentrations greater than 30µg/ml (50-80% inhibition). Therefore, these concentrations of antigen were considered to be toxic. The effect of lower nickel sulphate concentrations was variable; some cells were inhibited, other cells were stimulated.

Fig 3.1



PBM were incubated with 10µg/ml Con A and increasing concentrations of nickel sulphate in the 5 day LTT. The inhibition of cell proliferation to Con A was then determined.

(2) Time Course Studies and Dose Responses

Having established that nickel sulphate concentrations greater than 30μg/ml should not be used because of toxicity, time course and dose response studies were carried out to establish the optimal culture times and nickel sulphate concentrations to be used in further experiments. Initial experiments were carried out using 0-20μg/ml of nickel sulphate in 5-8 day assays.

Results from two experiments are shown in figs 3.2-3.5 and can be summarised as follows:-

Day 5 (fig 3.2):- Cells from nickel-sensitive patients exhibited a dose dependent response to nickel sulphate. There was no response from the controls' cells at concentrations of 0-10μg/ml of nickel sulphate; however, for one individual (C7, fig 3.2c), 20μg/ml was weakly mitogenic.

Day 6 (fig 3.3):- The response of the nickel-sensitive patients' cells increased. One control (C7) responded to 10 and 20μg/ml of nickel sulphate (fig 3.3c)

Day 7 (fig 3.4):- Nickel-sensitive patient lymphocytes were at maximum stimulation; however, the background values were increased (thus decreasing stimulation indices).

Day 8 (fig 3.5):- Lymphocyte transformation at higher nickel sulphate concentrations decreased.

Fig 3.2a

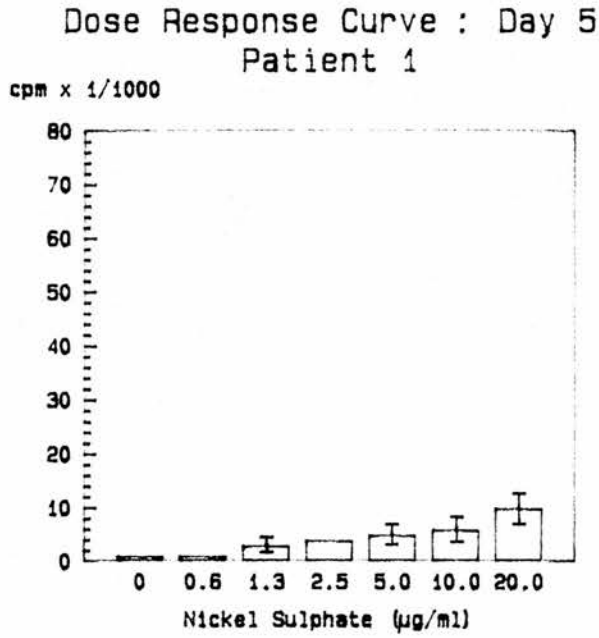


Fig 3.2b

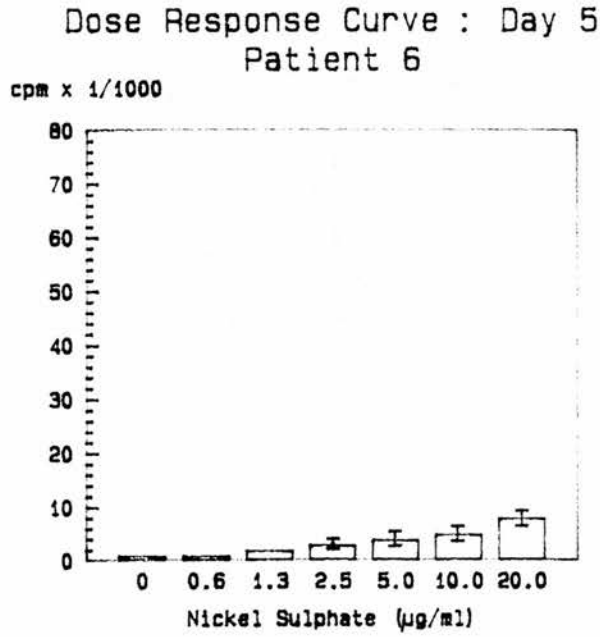


Fig 3.2c

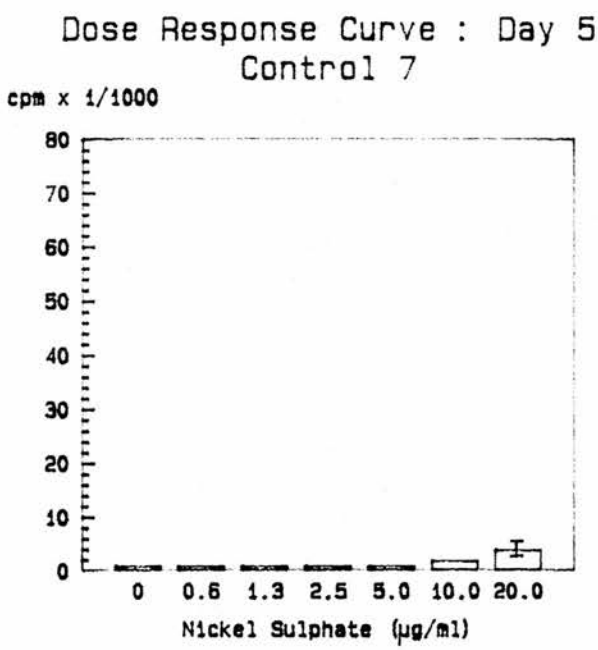
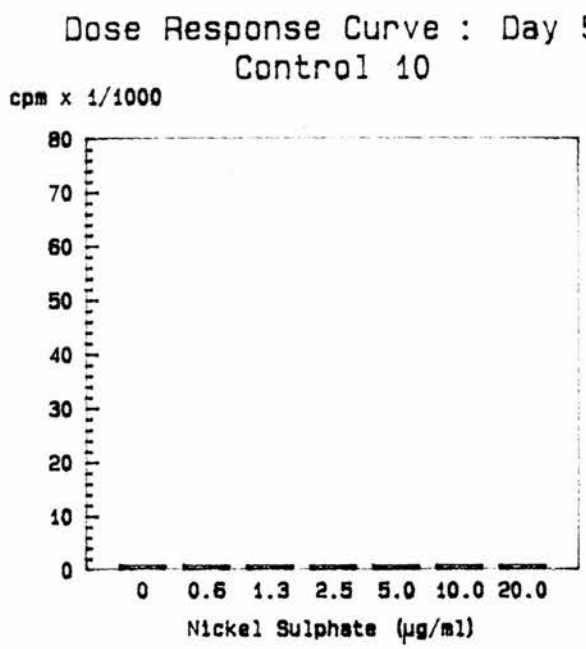


Fig 3.2d



PBM from 2 nickel-sensitive patients and 2 non-sensitised controls were incubated with 0-20µg/ml of $NiSO_4$ in a 5 day LTT. Results in cpm x 10^{-3} (mean ± SD)

Fig 3.3a

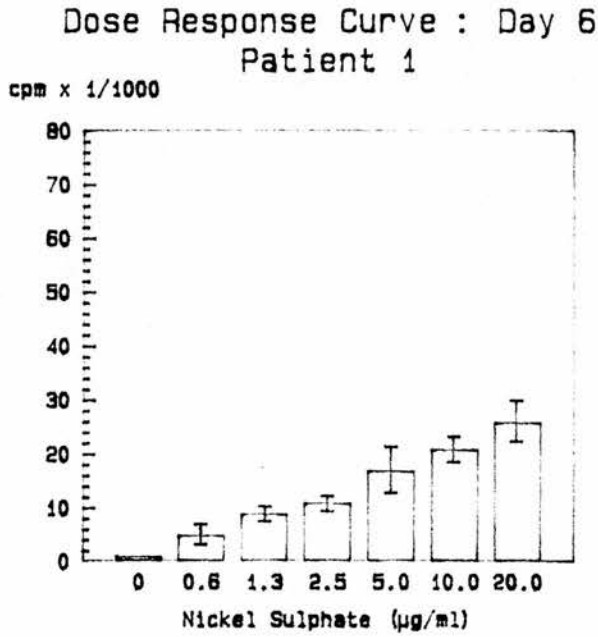


Fig 3.3b

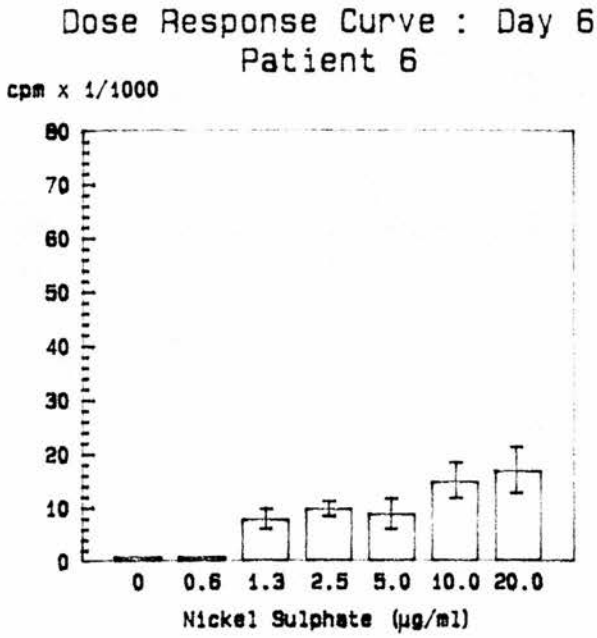


Fig 3.3c

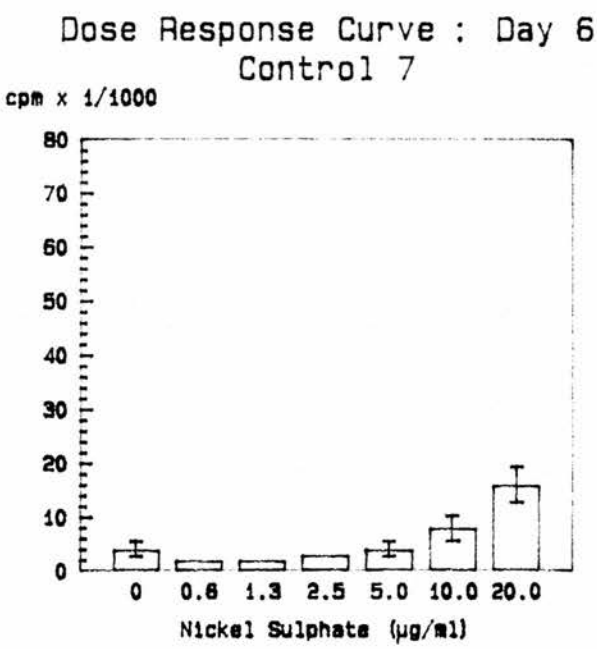
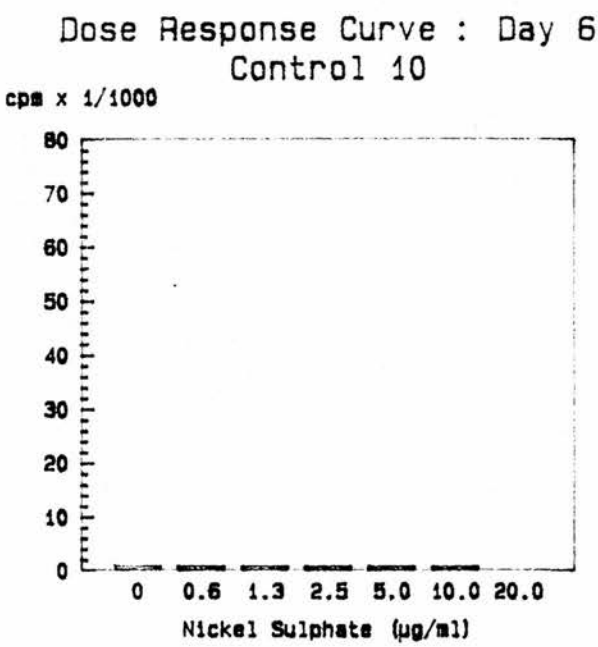


Fig 3.3d



PBM from 2 nickel-sensitive patients and 2 non-sensitised controls were incubated with 0-20µg/ml $NiSO_4$ in a 6 day LTT. Results in cpm x 10^{-3} (mean $\pm$ SD).

Fig 3.4a

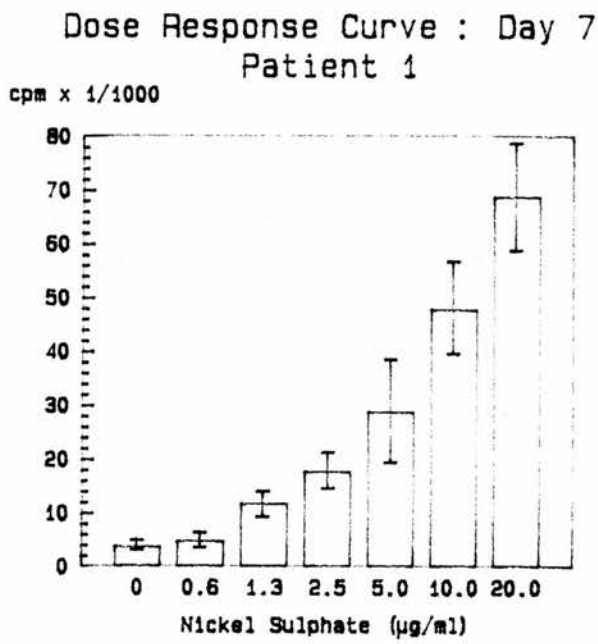


Fig 3.4b

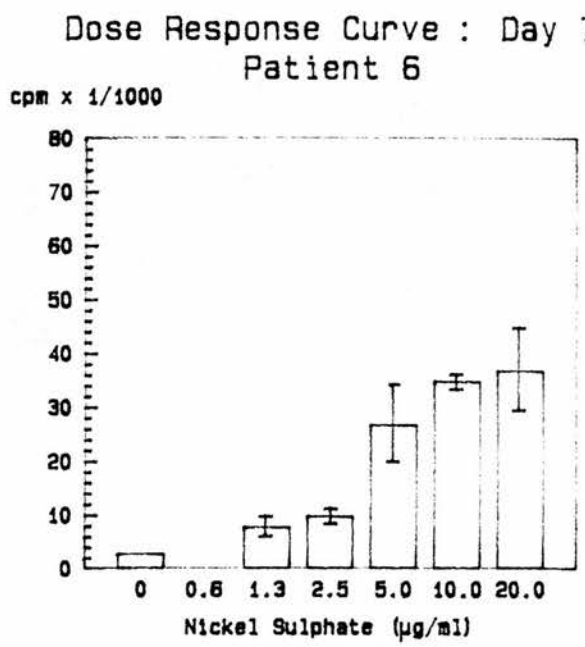


Fig 3.4c

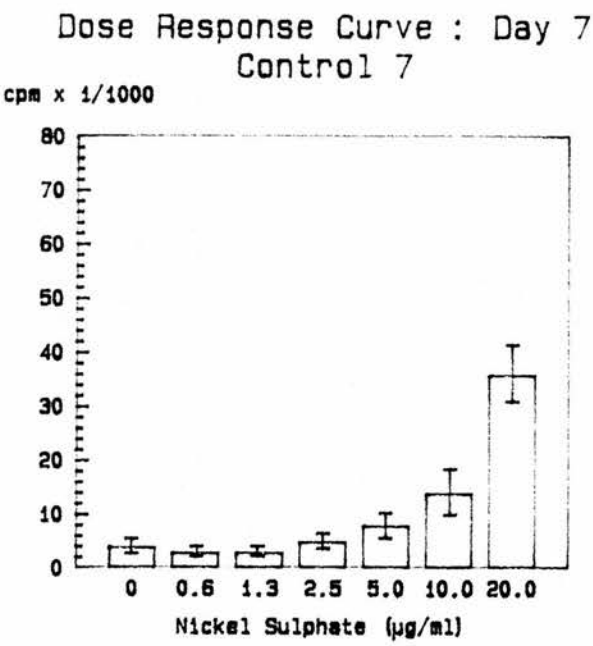
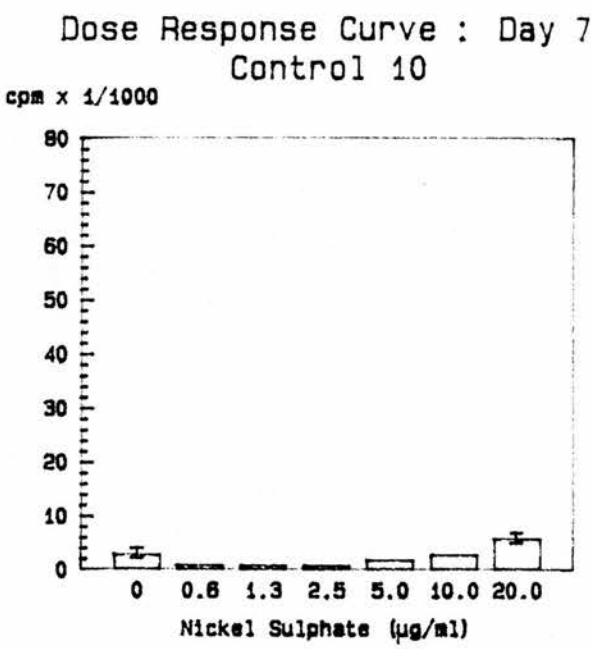


Fig 3.4d



PBM from 2 nickel-sensitive patients and 2 non-sensitised controls were incubated with 0-20µg/ml $NiSO_4$ in a 7 day LTT.
Results in cpm x 10^{-3} (mean $\pm$ SD)

Fig 3.5a

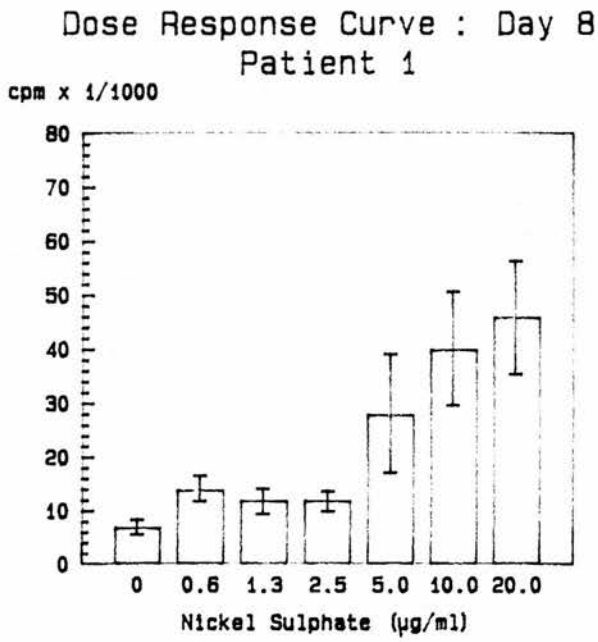


Fig 3.5b

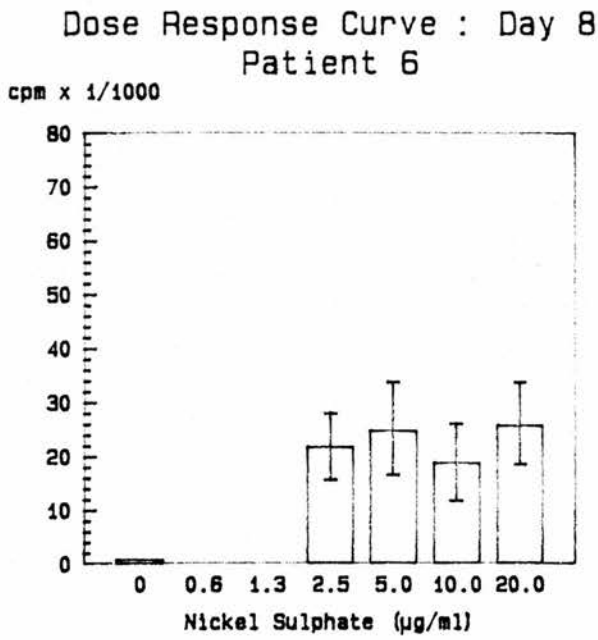


Fig 3.5c

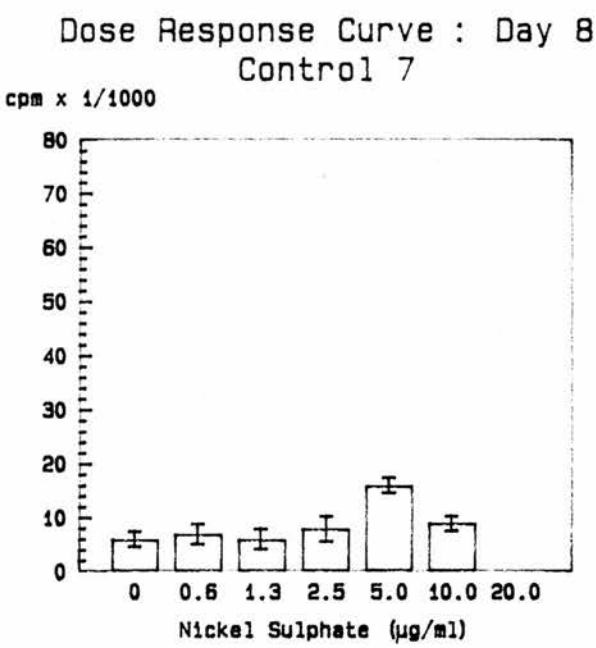
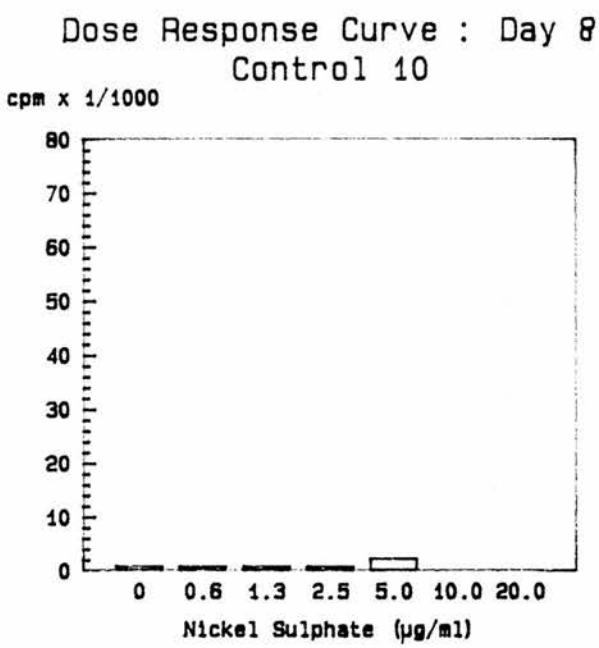


Fig 3.5d



PBM from 2 nickel-sensitive patients and 2 non-sensitised controls were incubated with 0-20µg/ml $NiSO_4$ in an 8 day LTT. Results in cpm x 10^{-3} (mean ± SD)

Time course curves for nickel sulphate concentrations of 2.5-20μg/ml are shown in fig 3.6. Stimulation indices are plotted, thus accounting for increasing background values. (The increased SI on day 8 (P6) resulted from a decreased background level). These initial experiments indicated that stimulation indices were maximal in 6 day assays. The stimulation index for control C7 was greater than 3 on day 6 when cells were incubated with 20μg/ml of nickel sulphate (fig 3.6a) and the SI was 2.5 with 10μg/ml (fig 3.6b). Therefore, 5μg/ml of nickel sulphate (SI approximately 1 for control cells) was chosen as a suitable concentration to discriminate between nickel-sensitive patients and controls using the LTT.

Fig 3.6

PBM were incubated with 0-20µg/ml $NiSO_4$ in 5, 6, 7 and 8 day LTTs. The SI (cells with $NiSO_4$ (cpm))/(cells in medium (cpm)) were calculated and plotted for each antigen concentration against culture time (days) (figs 3.6a-d).

Time Course Curves for Nickel Sulphate
20 µg/ml

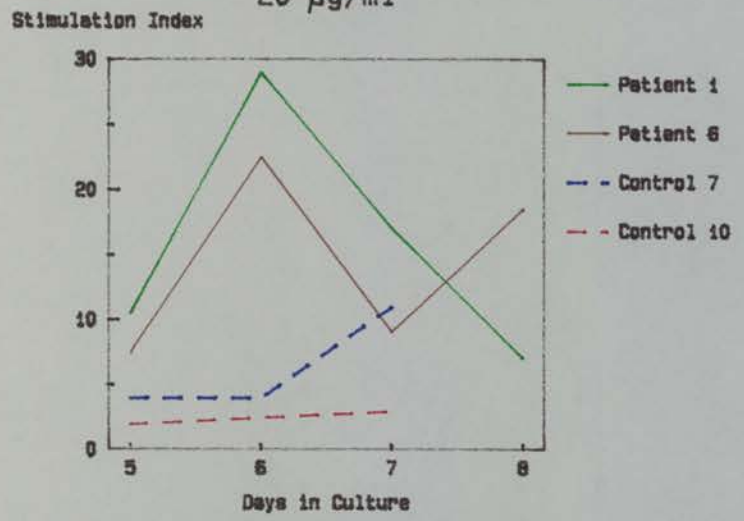


Fig 3.6a

Time Course Curves for Nickel Sulphate
10 µg/ml

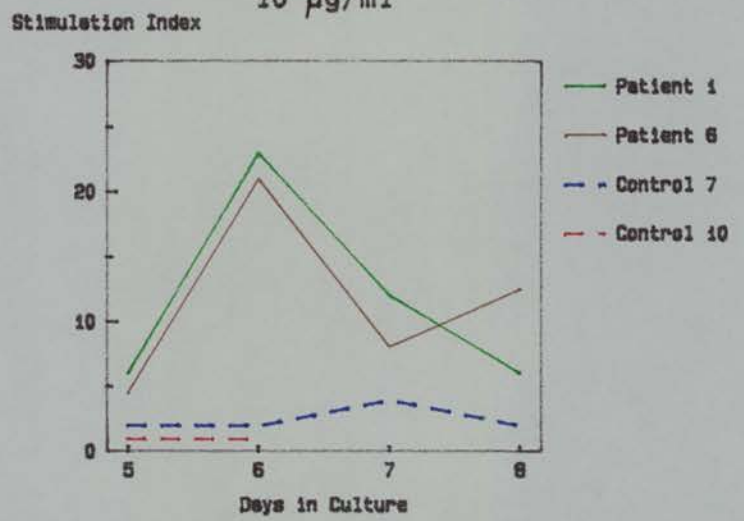


Fig 3.6b

Time Course Curves for Nickel Sulphate
5 µg/ml

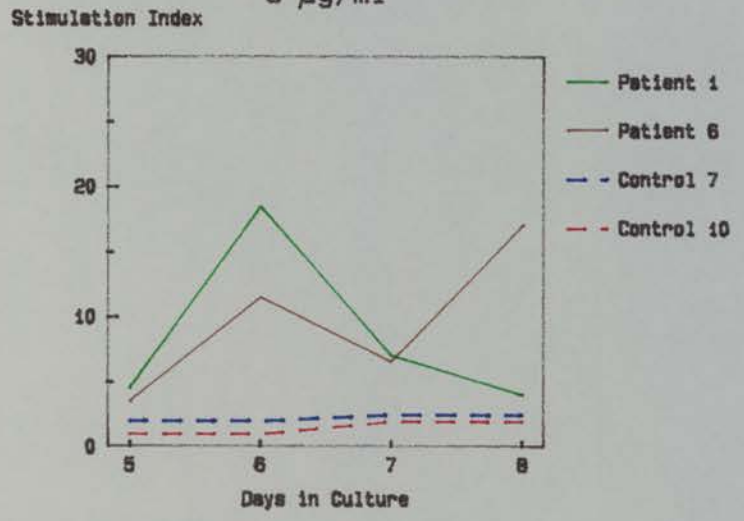


Fig 3.6c

Time Course Curves for Nickel Sulphate
2.5 µg/ml

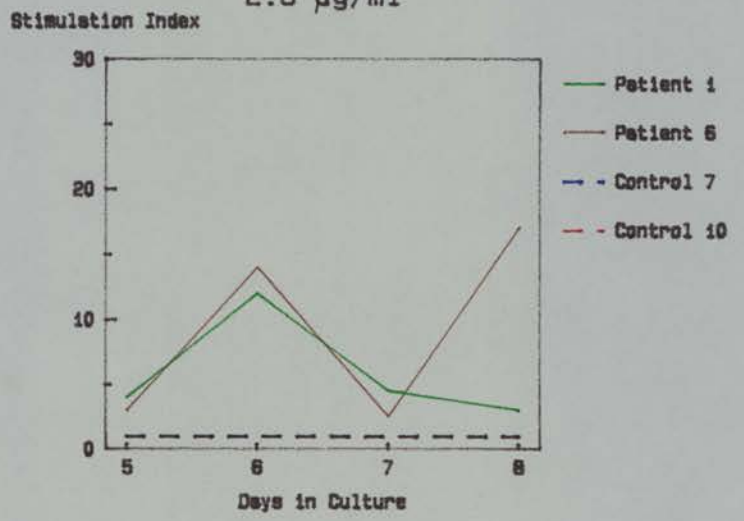
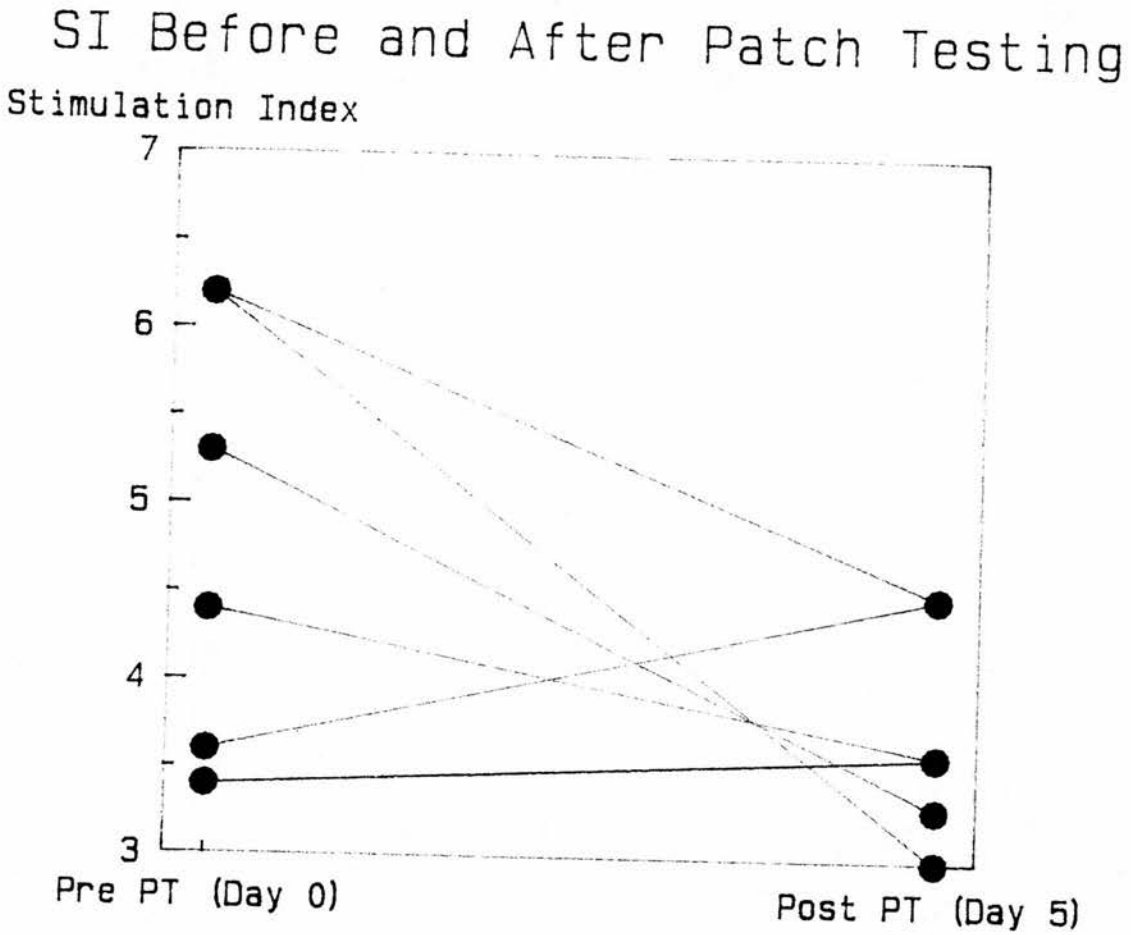


Fig 3.6d

THE EFFECTS OF PATCH TESTING ON THE LTT

A blood sample was taken from the majority of the nickel-sensitive patients when they attended the patch test clinic for their day 5 reading (ie 72 hours after patch removal). To ensure that patch testing was not influencing the outcome of the LTT, a blood sample was taken from six patients (P25, P33, P37, P47, P55 and P61) immediately before patch test application and when they attended the clinic for the day 5 reading. The stimulation indices are shown in fig 3.7; results obtained pre- and post-patch testing were not significantly different using Wilcoxon's Rank Sum Test ($p > 0.05$).

Fig 3.7



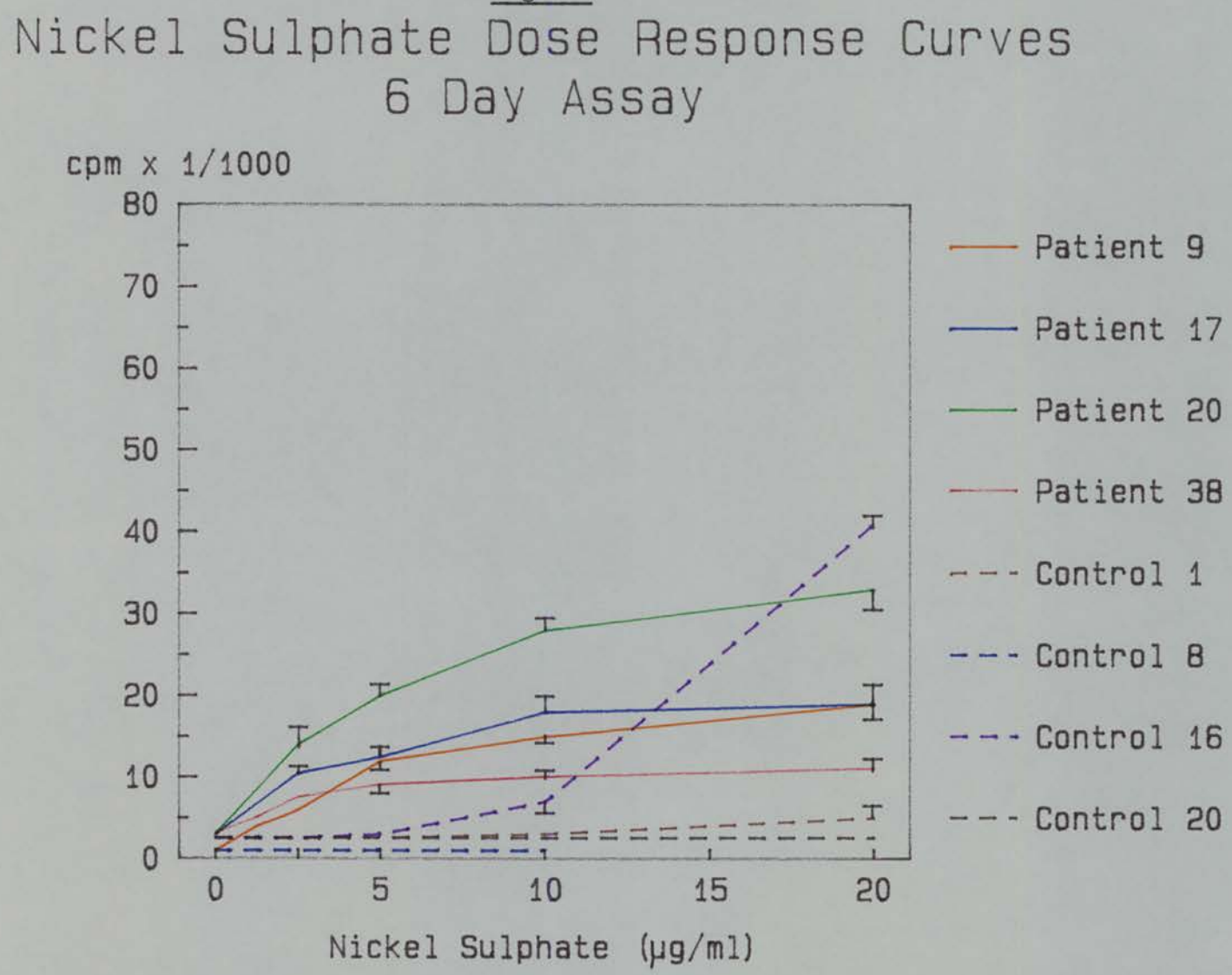
Two blood samples were taken from 6 patients when they attended the patch test clinic. The first was obtained before patch test application (day 0) and the second taken when they returned to the clinic for the day 5 reading. SI obtained using the two blood samples in the 6 day LTT were compared and were not significantly different ($p>0.05$; Wilcoxon's Rank Sum Test)

THE DETECTION OF NICKEL SENSITIVITY USING THE LTT

The 6 day LTT has been used to obtain dose response curves for PBM from 66 nickel-sensitive patients (58 females, 8 males, mean age=33) and 46 controls (32 females, 14 males, mean age=35). Peripheral blood mononuclear cells from 56 of the patients and 43 of the controls have also been studied in the 7 day LTT. Results are shown in Appendices III and IV (pages 178-86); representative results from 4 patients and 4 controls are shown in figs 3.8 and 3.9.

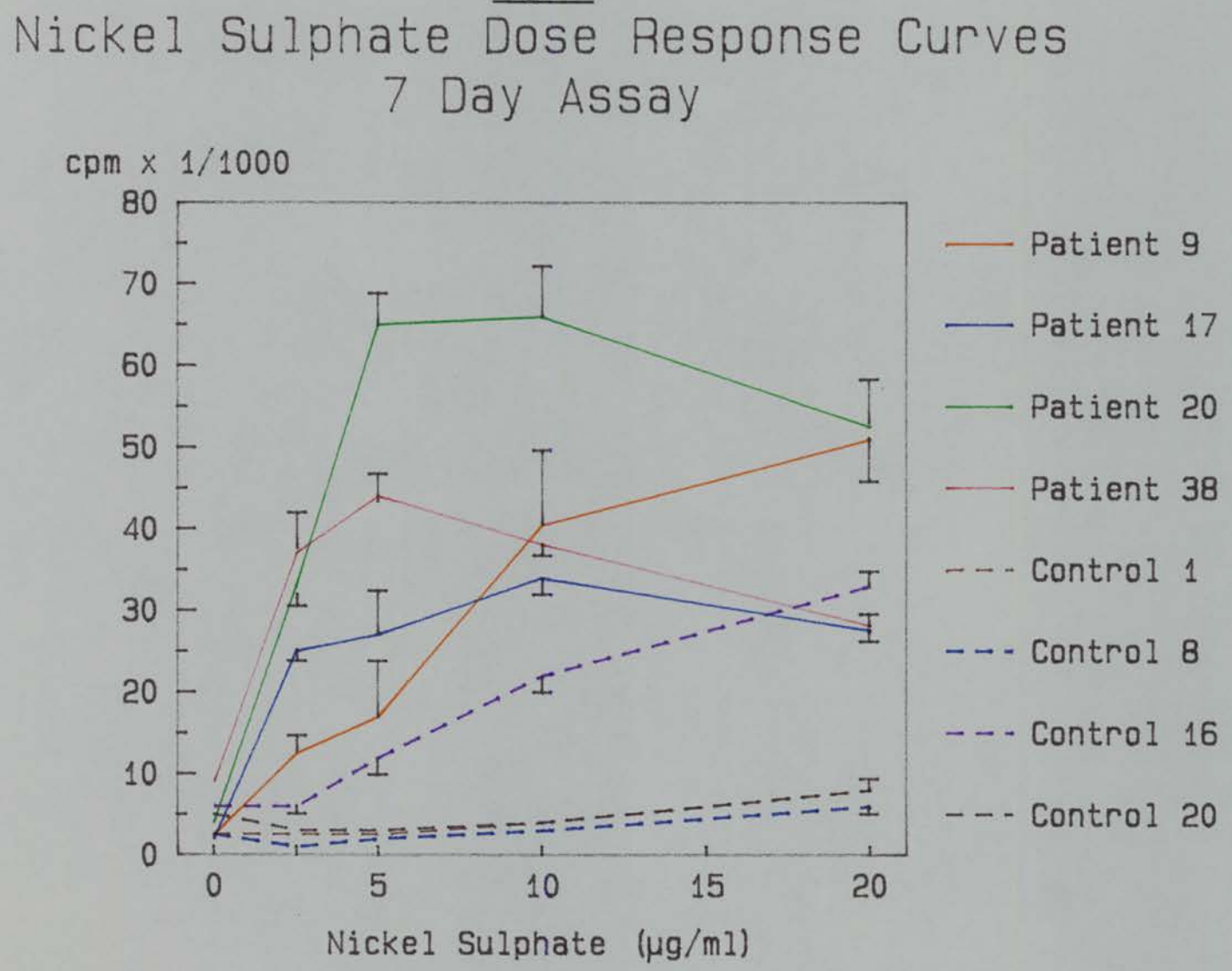
A stimulation index greater than or equal to 3 using 5µg/ml of nickel sulphate was chosen to discriminate between nickel-sensitive patients and controls (see page 71). Stimulation indices obtained from patients' cells using this antigen concentration are shown together with patch test results in Table 3.1; stimulation indices from control cells incubated with 5µg/ml of nickel sulphate are shown in Table 3.2.

Fig 3.8



Representative results obtained using PBM from 4 nickel-sensitive patients and 4 non-sensitised controls in the 6 day LTT (0-20µg/ml $NiSO_4$). Results in cpm x 10^{-3} (Mean ± SD).

Fig 3.9



Representative results obtained using PBM from 4 nickel-sensitive patients and 4 non-sensitised controls in the 7 day LTT (0-20µg/ml $NiSO_4$). Results in cpm x 10^{-3} (Mean ± SD).

Table 3.1 Patient Stimulation Indices Obtained With 5µg/ml $NiSO_4$ And Patch Test Results

Pat	Nickel P.T. Reaction		SI Day 6	SI Day 7	Pat	Nickel P.T. Reaction		SI Day 6	SI Day 7
P1	++	++	18.0	7.0	P2	++	+++	14.6	N.T.
P3	+++	+++	23.0	N.T	P4	++	++	29.0	6.1
P5	+++	+++	7.3	N.T	P6	++	++	11.0	9.8
P7	++	++	6.4	20.2	P8	-	+	9.0	71.0
P9	++	++	12.9	5.5	P10	+?	+	7.3	15.9
P11	++	+++	79.0	N.T.	P12	+	+	3.9	N.T.
P13	+	++	18.8	28.9	P14	+++	++	22.0	N.T.
P15	N.A.	N.A.	7.1	N.T	P16	++	++	2.1	4.7
P17	++	++	3.6	7.9	P18	++	++	5.6	N.T.
P19	++	++	3.1	10.7	P20	++	++	7.3	15.4
P21	+	+	3.3	5.8	P22	++	++	3.5	5.6
P23	+	++	19.2	14.1	P24	-	++	1.9	3.2
P25	++	++	6.3	16.4	P26	++	++	6.3	2.5
P27	+++	+++	28.2	29.0	P28	++	++	19.6	15.2
P29	+++	+++	15.9	13.0	P30	+	++	2.9	4.3
P31	+?	++	3.0	3.5	P32	+?	+	2.4	4.2
P33	+?	+	3.4	3.2	P34	-	+	5.4	3.2
P35	+	+	3.0	3.6	P36	-	+	3.6	3.0
P37	++	++	4.5	4.9	P38	+	++	3.1	4.8
P39	++	++	3.7	5.6	P40	++	++	5.5	N.T.
P41	+++	+++	3.2	N.T.	P42	+	++	3.3	5.4
P43	+	++	4.4	10.0	P44	+	++	4.6	4.3
P45	+	++	3.6	4.8	P46	+	++	6.3	2.5
P47	+++	++	4.4	6.4	P48	+++	+++	6.5	N.T.
P49	N.A.	++	2.4	3.0	P50	+	++	4.1	3.4
P51	+++	+++	12.6	13.8	P52	+++	+++	2.1	1.9
P53	+	++	2.1	2.9	P54	++	++	12.0	11.9
P55	+	++	5.3	3.3	P56	+?	+	2.3	2.7
P57	N.A.	N.A.	2.5	5.3	P58	+	+	2.7	2.1
P59	-	+	4.5	3.3	P60	+	+	3.3	3.5

Table 3.1 contd

Pat	Nickel P.T. Reaction		SI Day 6	SI Day 7	Pat	Nickel P.T. Reaction		SI Day 6	SI Day 7
P61	N.A.	N.A.	7.3	4.4	P62	+	++	3.4	9.7
P63	++	++	5.0	3.4	P64	+	+	1.8	1.4
P65	+	+	4.5	7.8	P66	+++	+++	21.2	11.6

N.A. = Not Available (but known to be positive)

N.T.= Not Tested

Table 3.2 Control Stimulation Indices Obtained Using 5µg/ml $NiSO_4$

Cont	SI Day 6	SI Day 7	Cont	SI Day 6	SI Day 7	Cont	SI Day 6	SI Day 7
C1	1.0	0.5	C2	0.7	0.8	C3	2.0	2.4
C4	1.5	1.5	C5	0.7	0.6	C6	0.5	0.9
C7	1.0	2.5	C8	0.5	0.7	C9	0.5	1.4
C10	1.3	0.4	C11	1.5	1.9	C12	2.1	1.8
C13	1.1	2.7	C14	2.4	1.2	C15	2.5	0.8
C16	0.7	2.1	C17	2.2	2.0	C18	1.9	1.4
C19	0.3	N.T.	C20	0.9	0.6	C21	2.0	2.5
C22	1.3	1.4	C23	1.2	1.5	C24	1.3	1.0
C25	1.3	1.3	C26	0.9	N.T.	C27	2.6	N.T.
C28	0.8	2.2	C29	1.0	1.0	C30	1.2	1.8
C31	1.2	1.3	C32	1.7	1.8	C33	0.9	0.5
C34	1.3	1.5	C35	1.3	0.9	C36	1.0	1.4
C37	0.8	1.3	C38	2.0	1.3	C39	1.3	1.5
C40	1.3	1.7	C41	1.1	1.3	C42	0.7	1.4
C43	1.8	1.7	C44	0.3	1.6	C45	2.0	2.2
C46	1.2	2.4						

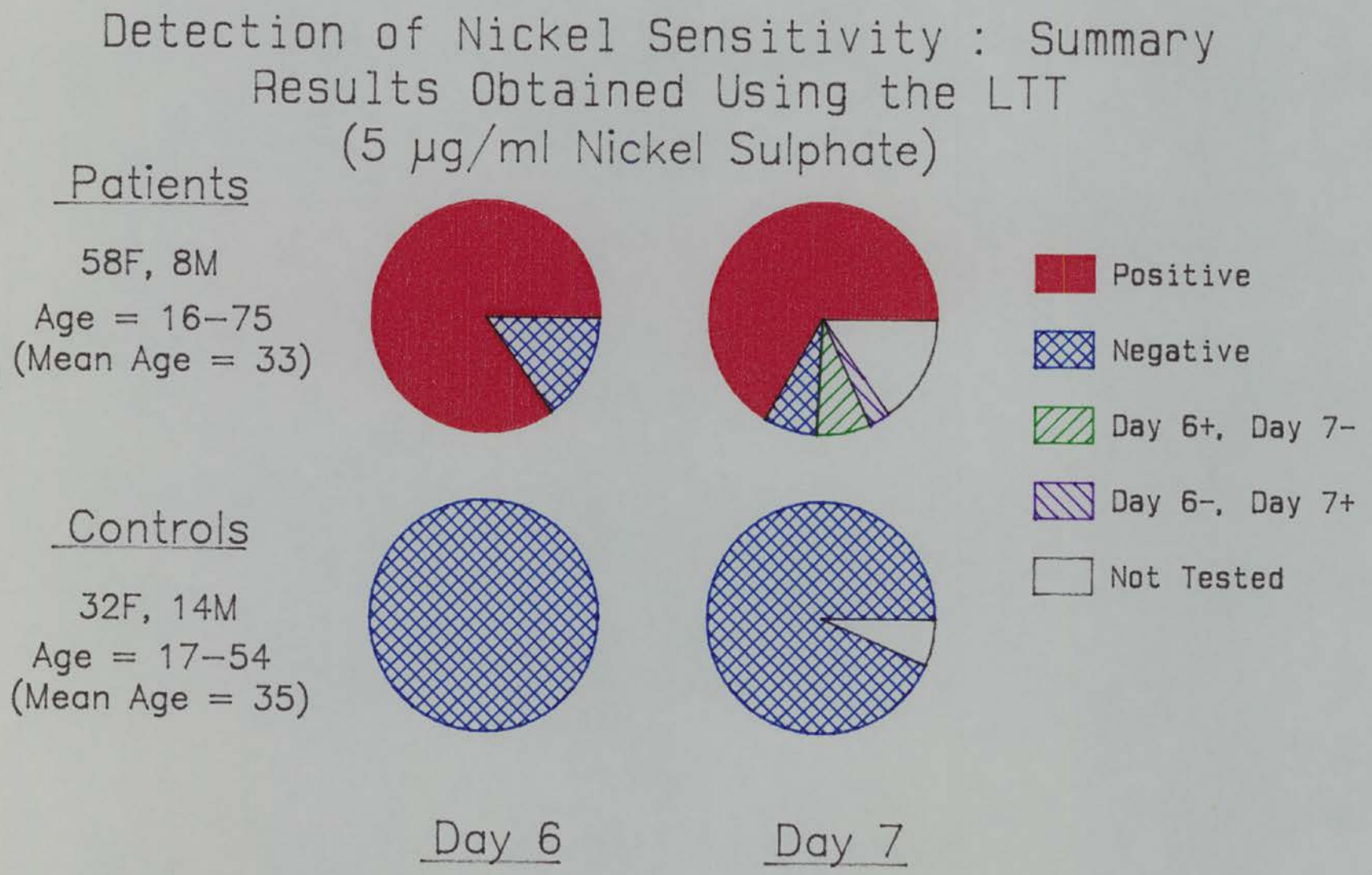
Employing the criterion of an SI$\geqslant$3 as a positive response in the LTT, 56/66 patients were positive in the 6 day assay and 49/56 in the 7 day assay (fig 3.10). Five patients (P16, P24, P30, P32 and P49) were positive only in the 7 day assay; two patients (P26 and P46) were positive in the 6 day assay and not the 7 day LTT. Together, the 6 and 7 day assays detected 61/66 (92%) of the nickel-sensitive patients. None of the controls (0/46) were positive in the LTT.

The patients not detected by the 6 and 7 day assays were P52, P53, P56, P58 and P64. Three of these patients (P56, P58 and P64) had weak positive patch test reactions to nickel sulphate. Patient P52 gave a strong patch test reaction, but may not have been detected in the 7 day assay because of the high background value obtained (See Appendix III, page 181). Cells from all 5 patients responded against Con A (See Appendix III, pages 181-82).

The significance of differences between patient and control cells were determined using the student's t-test and are shown in Table 3.3.

As expected, there were no statistical differences between patient and control cells incubated in medium (background proliferation) ($0.5 > p > 0.1$) and when the cells were incubated with Con A ($p > 0.5$). Significant differences were obtained for nickel sulphate concentrations between 2.5 and 20µg/ml ($p < 0.001$) in both the 6 and 7 day assays.

Fig 3.10



Summary of the numbers of nickel-sensitive patients and non-sensitised controls positive and negative in the 6 and 7 day LTTs using 5µg/ml $NiSO_4$.

Table 3.3 Significance of Differences Between Patient and Control Cell Stimulation

	DAY 6			DAY 7		
Stimulant	t	df	p	t	df	p
Medium	0.718	110	0.1-0.5	0.360	97	> 0.5
10µg/ml Con A	0.668	110	> 0.5	0.384	95	> 0.5
20µg/ml $NiSO_4$	5.73	93	< 0.001	5.76	86	< 0.001
10µg/ml $NiSO_4$	8.15	105	< 0.001	7.24	97	< 0.001
5µg/ml $NiSO_4$	8.56	110	< 0.001	7.46	97	< 0.001
2.5µg/ml $NiSO_4$	7.09	109	< 0.001	6.69	93	< 0.001

CORRELATION BETWEEN PATCH TEST AND LTT RESULTS

The Wilcoxon's Rank Sum Test was used to evaluate the correlation between patch test scores and stimulation indices obtained in the the LTT using 5µg/ml of nickel sulphate (shown in Table 3.1).

The correlation between the patch test scores and the *in vitro* results was weak. Correlation coefficients of 0.38 and 0.33 were obtained for patch test scores with 6 and 7 day results respectively.

ACCESSORY CELL FUNCTION OF BLOOD AND EPIDERMAL CELLS

Peripheral blood mononuclear cells include T-cells, B-cells, monocytes and dendritic cells. To establish the ability of different cell types to act as accessory cells it was first necessary to obtain a highly enriched T-cell population with insignificant accessory cell contamination and consequently incapable of proliferation in the presence of mitogen or antigen.

Monocytes/macrophages can be isolated by their ability to adhere readily to plastic or glass surfaces. In addition, dendritic cells also adhere to surfaces during the first two hours of incubation, but become detached after 18 hours.

Two methods are commonly used to purify T-cells:-
rosetting with sheep erythrocytes or
passage through nylon wool columns.

Rosetting

In the early 1970's, it was discovered that some human lymphocytes bind *in vitro* to sheep red blood cells (SRBC) to form rosettes . It was suggested that the rosette-forming cells were T-cells (Wybran and Fudenberg, 1971). In 1973, Weiner *et al.* reported that neuraminidase treatment of SRBC enhanced rosetting activity and a year later, Kaplan and Clark (1974) described another improved procedure for rosetting T-cells by pre-treating the SRBC with a sulphydryl reagent 2-aminoethyisothiouronium bromide (AET). After rosetting, the cells are subjected to osmotic shock to lyse the SRBC.

Nylon Wool Passage

The isolation of murine T-cells by the passage of lymphocytes through nylon fibre columns at 37^{o}C was described by Julius *et al.* (1973). B-cells adhere to the nylon fibres and are retained in the column.

Nylon wool passage has two advantages over SRBC rosetting. Firstly, the enriched T-cells are not subjected to osmotic shock, and secondly, the T-cells will not be activated by binding at the SRBC receptor (CD2).

Enrichment of T-Cells

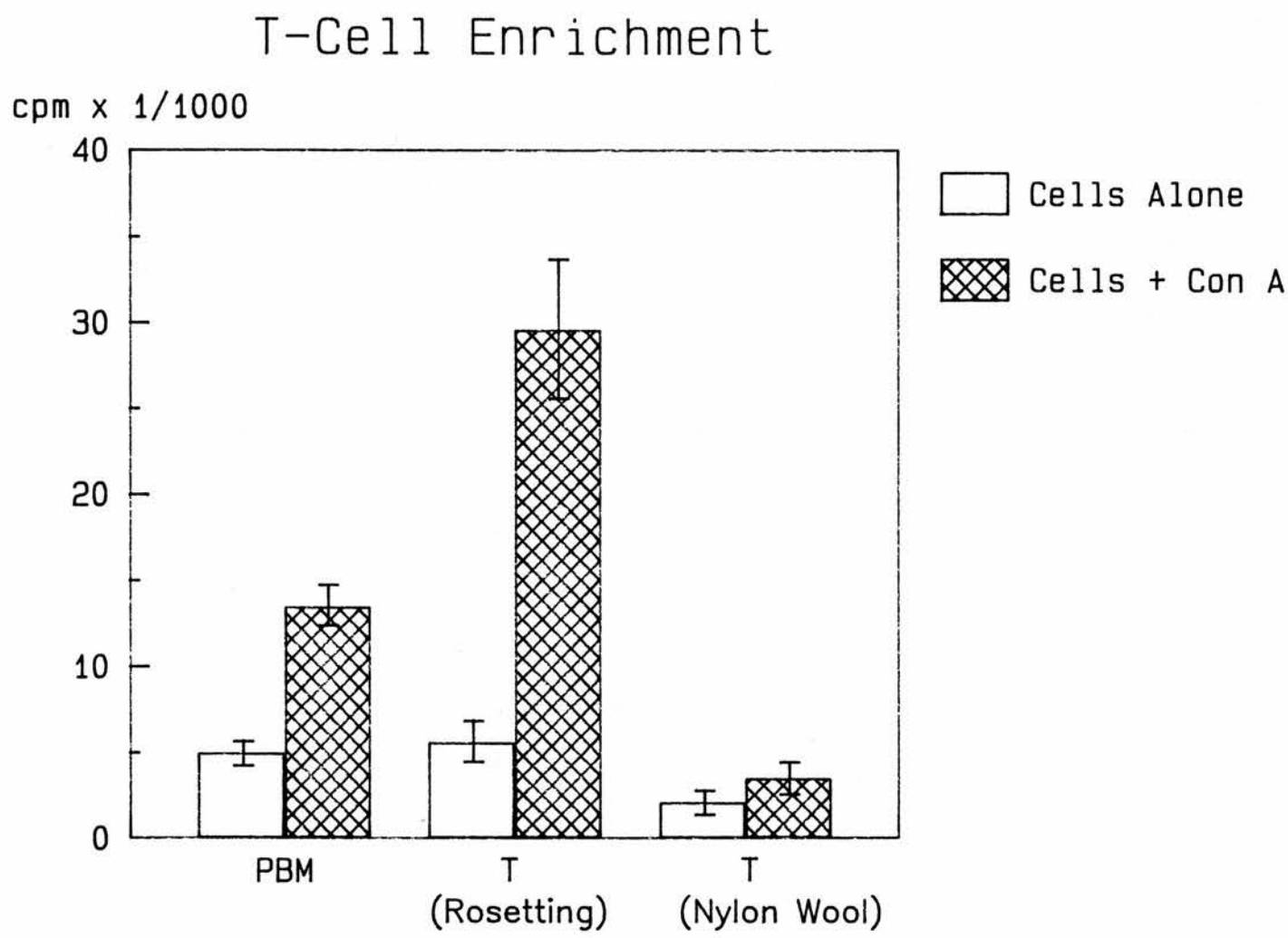
AET-SRBC rosetting and nylon wool passage were compared in order to assess the most suitable method of T-cell enrichment for studies of accessory cell function.

PBM were obtained from blood samples and the plastic-adherent blood cells were removed (as described on page 52). The plastic-non-adherent cells were divided into two equal groups. T-cells were obtained from cells which either were incubated on a nylon wool column for 1 hour ($37^{o}C$) and then incubated on glass for 2½ hours at $37^{o}C$ (page 55), or from cells which were rosetted with AET-SRBC, isolated from the non-rosetted cells and then subjected to osmotic shock (to lyse the SRBC), as described on pages 53-54.

T-cells were then incubated with 10µg/ml Con A in a 5 day proliferation assay. Results (representative of 3 experiments) are shown in fig 4.1.

As fig 4.1 shows, the T-cells obtained using the rosetting procedure responded to Con A in the absence of additional accessory cells whereas the T-cells obtained from passage through nylon wool did not. Therefore, nylon wool passage followed by glass adherence was used as the method of choice in enriching T-cells.

Fig 4.1



Plastic-non-adherent cells were obtained from PBM and divided into 2 groups. One group was incubated on a nylon wool column for 1 hour and then on glass for 2½ hours (T Nylon Wool). T-cells were isolated from the other group by rosetting with AET-SRBC, separation of rosettes using centrifugation on lymphocyte separation medium and lysis of the SRBC by osmotic shock (T Rosetting). PBM and 'enriched T-cells' were incubated with Con A in the 5 day LTT. Response against the mitogen by T-cells indicated the presence of contaminating accessory cells. Results in cpm x 10^{-3} (mean $\pm$ SD).

Characterisation of Cell Populations

Antigen presentation requires the presence of MHC II^+ cells. Therefore, to ensure that the T-cell populations obtained were highly enriched and that cells capable of antigen presentation had been removed, cells were stained using indirect immunofluorescence for the expression of MHC II antigens using antibody DA6.231 (anti HLA-DP, -DQ and -DR) (see page 57). The percentages of cells expressing MHC class II antigens are summarised in Table 4.1.

Table 4.1 The Expression of MHC II Antigens By PBM, Plastic-Adherent Blood Cells, Enriched T-Cells and Epidermal Cells

Cell Population	n	$DA6.231^+$ Cells	
		Range(%)	Mean(%)
PBM	22	7.5-16	11.3
Plastic-Adherent	22	28-72	54.6
Nylon Wool Enriched T	22	0-1.0	0.4
Epidermal	6	1.2-3.0	2.2

Previous authors including Braathen (1980) have stated that enriched T-cells should contain < 1% macrophages and < 5% B-cells. In the experiments described in this thesis, if > 0.5% T-cells expressed MHC II antigens, the T-cells were considered to be not fully enriched.

The Ability Of Plastic-Adherent Blood Cells To Act As Accessory Cells

The ability of plastic-adherent blood cells to act as accessory cells in the presence of 10μg/ml of Con A and 0-10μg/ml of nickel sulphate in a 6 day LTT was studied in 18 experiments using cells from 15 nickel-sensitive patients and in 16 experiments using cells from 8 non-sensitised controls. Nylon wool enriched T-cells (10^6/ml) were incubated with Con A or 5μg/ml of nickel sulphate in the presence or absence of 10^5/ml (10%) plastic-adherent blood cells. The results were then compared with those obtained using 10^6/ml PBM. The data are shown in Appendices V and VI (pages 187-89). Stimulation indices obtained using Con A and 5μg/ml $NiSO_4$ are shown in Tables 4.2 and 4.3.

Stimulation of nylon wool enriched T-cells (SI $\geqslant$3) by Con A (column 3) or nickel sulphate (column 6) from patients P4b, P20b, P48a, P51 and P62 (fig 4.2) indicated that in these experiments the T-cells were not fully enriched. Patient T-cell responses against Con A and nickel sulphate were reconstituted by the addition of irradiated plastic-adherent blood cells in 15/18 experiments (eg P50, fig 4.3). There was no reconstitution of the Con A response by plastic-adherent blood cells from P41 or reconstitution of the antigen-specific response by cells from P4a. There was no reconstitution of both the mitogen- and antigen-specific response using cells from P9 (fig 4.4) and it is possible that the plastic-adherent cells from this patient had lost their accessory cell function during their preparation even though they had suffered no loss of viability.

A response against Con A was evident in the nylon wool enriched T-cells from controls reconstituted with plastic-adherent blood cells in all of the experiments, with one exception (C27) which was due to high background proliferation (see Appendix VI, page 189). T-cell proliferation in the presence of 5µg/ml of nickel sulphate was absent in all 16 experiments using cells from controls (Table 4.3 columns 5-7; eg C13a, fig 4.5).

Using a paired t-test, stimulation indices for PBM and T-cells reconstituted with plastic-adherent blood cells, and stimulated with either Con A or nickel sulphate were not significantly different.

Table 4.2

Stimulation Indices Obtained Using Con A and 5μg/ml Nickel Sulphate With PBM And Nylon Wool Enriched T-Cells With Or Without Added Plastic-Adherent Blood Cells

Patients	10μg/ml Con A			5μg/ml $NiSO_4$		
Pat	PBM	T	T+10%Ad	PBM	T	T+10%Ad
P1	6.0	1.2	3.6	4.1	N.T.	3.2
P3	70.0	2.5	102.0	23.0	0.5	54.0
P4a	102.0	2.0	15.3	29.0	1.0	1.4
P4b	22.5	3.5	22.0	7.3	1.5	14.0
P9	11.4	1.6	2.8	4.2	0.5	1.8
P12	3.4	0.5	3.5	3.0	0.5	3.9
P14	21.0	2.0	3.4	22.0	0.8	54.0
P18	2.7	1.3	3.1	4.5	1.0	8.3
P20a	5.2	1.2	4.3	5.2	0.5	9.0
P20b	4.4	1.6	3.6	8.9	3.8	12.3
P40	9.7	2.2	6.2	10.8	N.T.	16.6
P41	3.3	1.3	2.1	3.2	N.T.	3.6
P46	3.5	1.6	8.4	3.8	0.9	3.7
P48a	6.7	2.0	6.3	6.5	5.0	8.3
P48b	6.1	1.3	5.7	6.2	1.7	6.2
P50	22.9	2.0	13.6	4.1	1.7	6.0
P51	20.8	2.0	17.5	12.5	7.0	12.6
P62	28.8	14.0	42.9	9.1	10.1	13.7

N.T. = Not Tested

Table 4.3

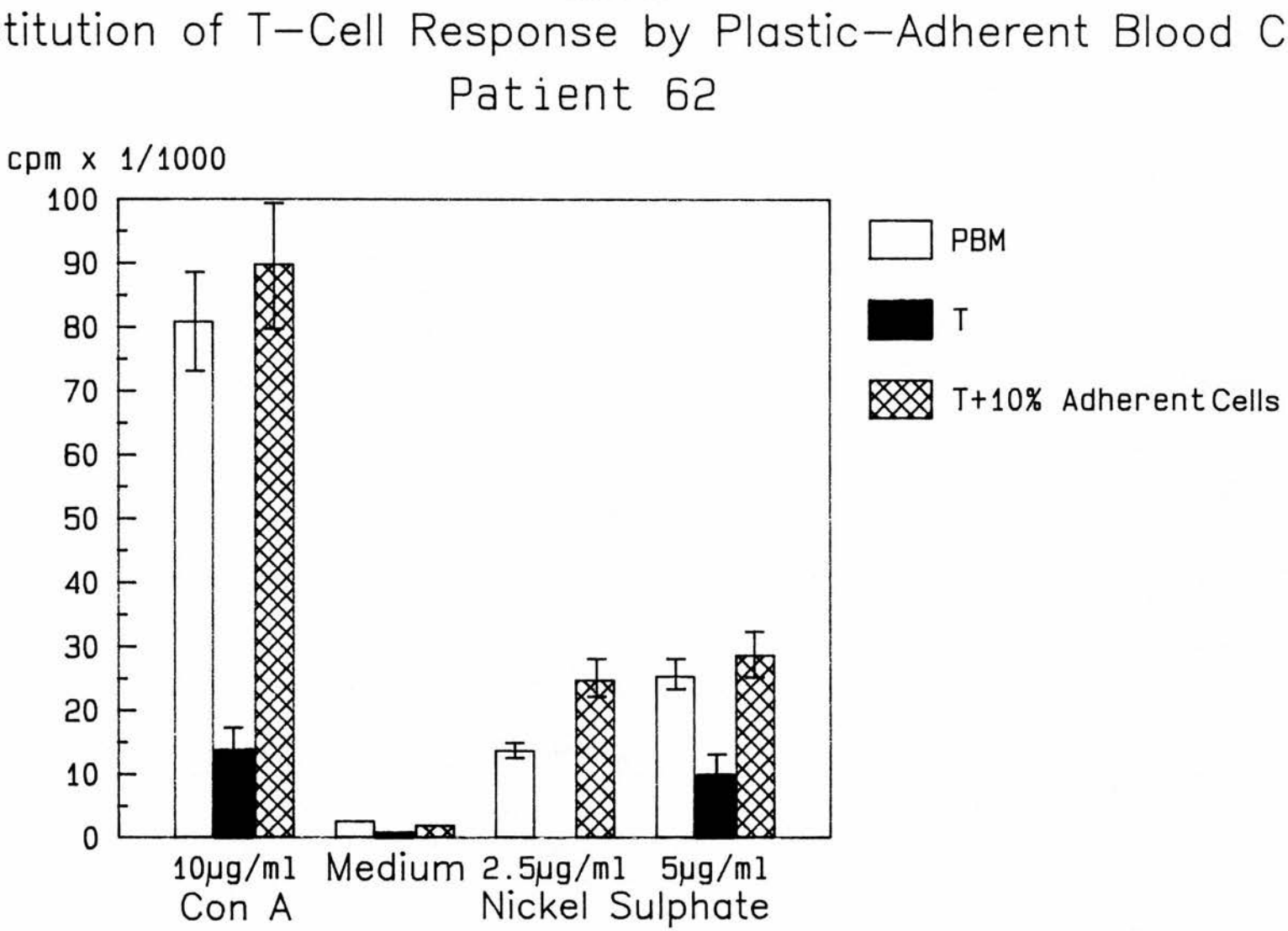
Stimulation Indices Obtained Using Con A And 5µg/ml Nickel Sulphate With PBM And Nylon Wool Enriched T-Cells With Or Without Added Plastic-Adherent Blood Cells

Controls

	10µg/ml Con A			5µg/ml $NiSO_4$		
Cont	PBM	T	T+10%Ad	PBM	T	T+10%Ad
C7	88.0	1.3	9.6	1.0	N.T.	1.3
C11a	5.8	1.7	6.6	1.2	0.8	1.1
C11b	6.0	1.4	4.4	1.3	1.4	1.3
C11c	8.5	1.9	11.7	2.0	0.5	2.1
C11d	9.0	2.5	8.1	2.8	1.3	1.6
C12a	3.8	2.5	4.9	1.3	N.T.	1.1
C12b	7.4	1.4	5.4	2.7	N.T.	2.7
C12c	19.3	1.5	13.2	1.4	1.0	1.6
C13a	20.6	1.7	19.5	2.3	2.2	2.2
C13b	10.6	1.8	6.4	1.5	N.T.	1.1
C17	76.0	1.1	9.1	1.0	0.8	0.7
C26a	5.6	1.6	9.4	0.9	N.T.	2.7
C26b	9.9	1.1	9.8	2.0	0.8	2.9
C26c	5.3	1.1	4.6	1.8	0.5	1.0
C27	5.4	1.1	2.3	2.6	0.5	1.4
C31	18.3	1.7	30.0	0.4	1.7	1.3

N.T. = Not Tested

Fig 4.2

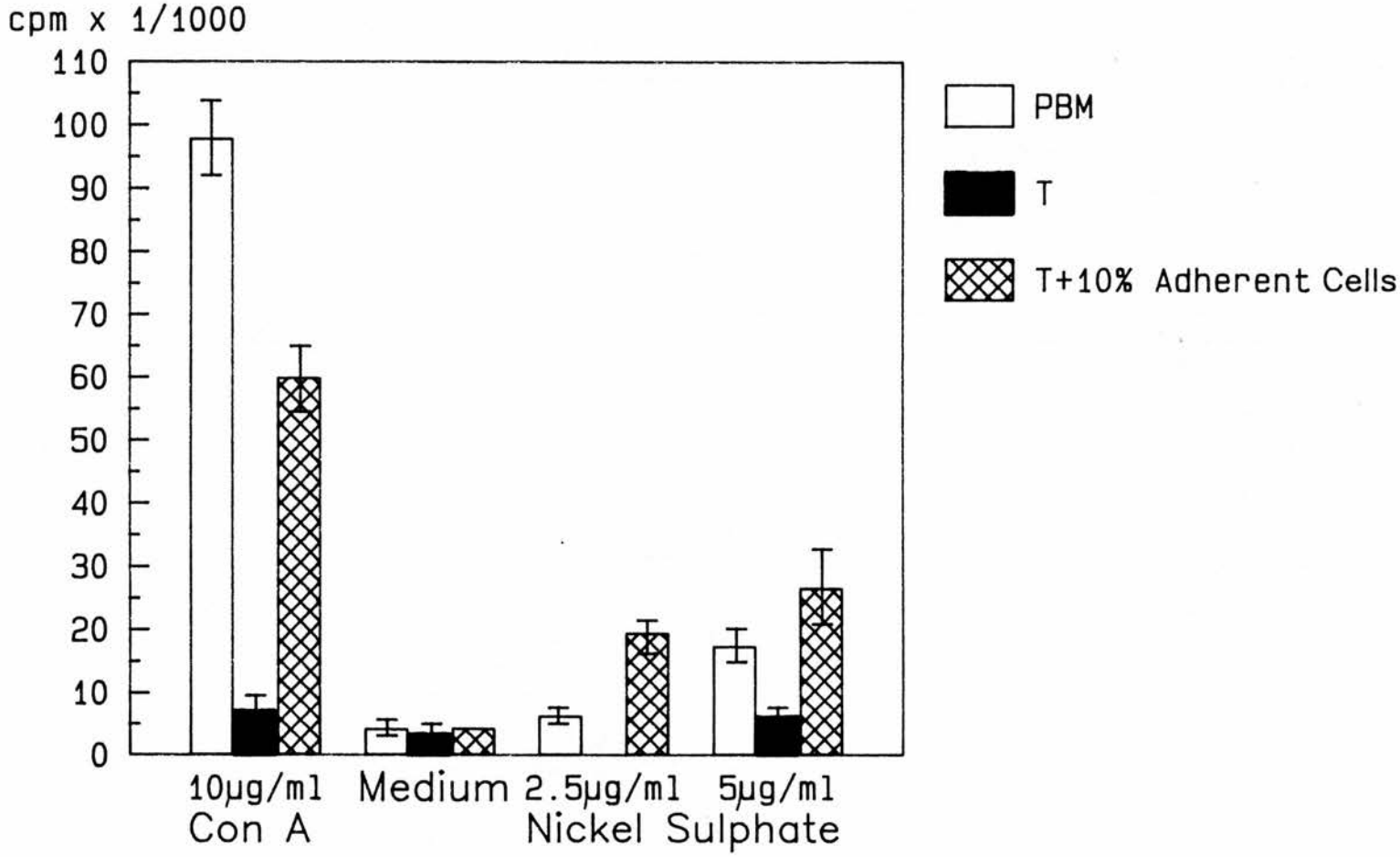


Stimulation of PBM, nylon wool enriched T-cells, and nylon wool enriched T-cells with added irradiated plastic-adherent blood cells (10%) from a nickel-sensitive patient using medium, 10µg/ml Con A and 2.5 µg/ml and 5µg/ml $NiSO_4$ in a 6 day LTT. Results in cpm x 10^{-3} (Mean ± SD).

Fig 4.3

Reconstitution of T–Cell Response by Plastic–Adherent Blood Cells

Patient 50

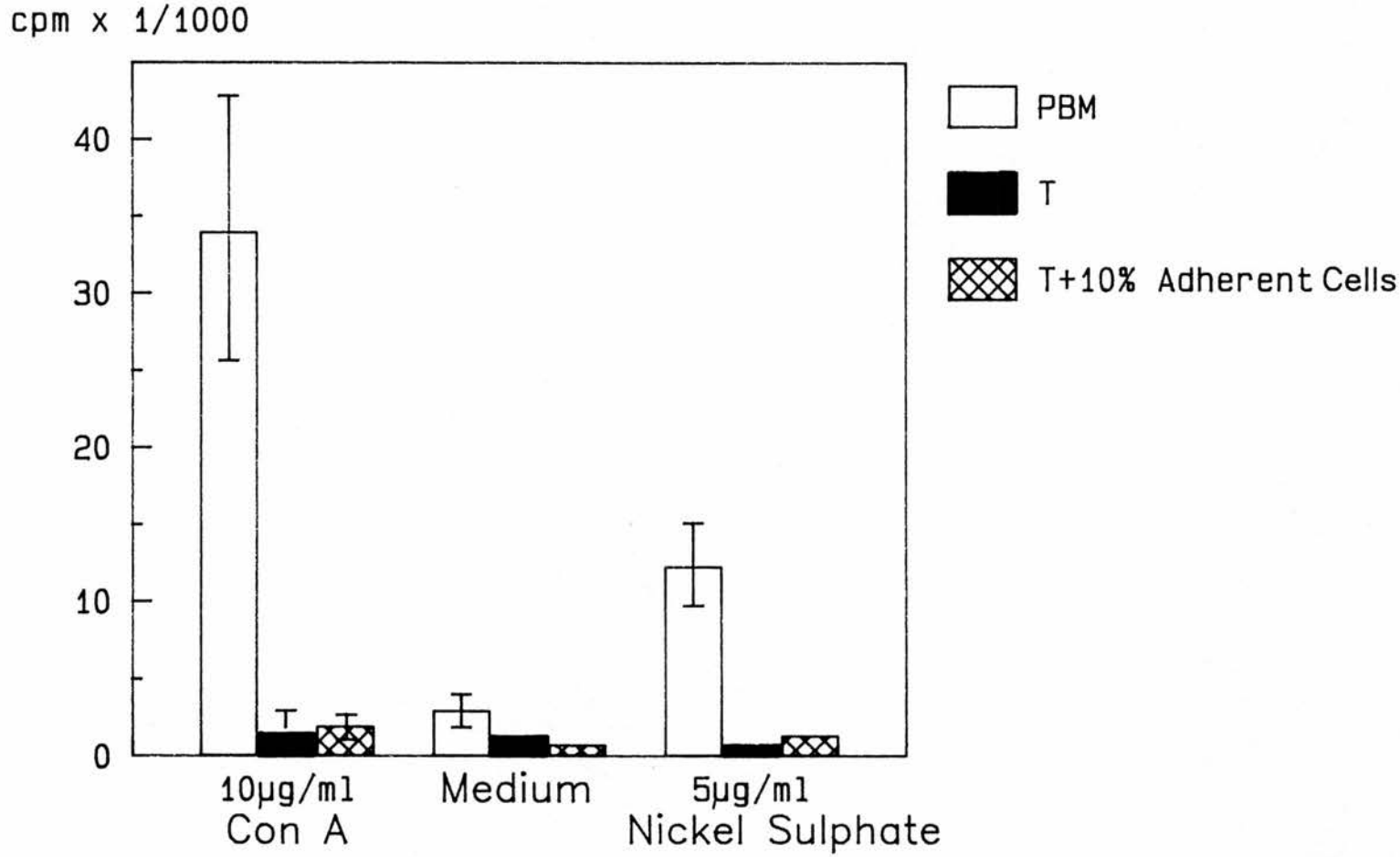


Stimulation of PBM, nylon wool enriched T-cells, and nylon wool enriched T-cells with added irradiated plastic-adherent blood cells (10%) from a nickel-sensitive patient using medium, 10µg/ml Con A and 2.5µg/ml and 5µg/ml $NiSO_4$ in a 6 day LTT. Results in cpm x 10^{-3} (Mean ± SD).

Fig 4.4

Reconstitution of T-cell Response by Plastic-Adherent Blood Cells

Patient 9

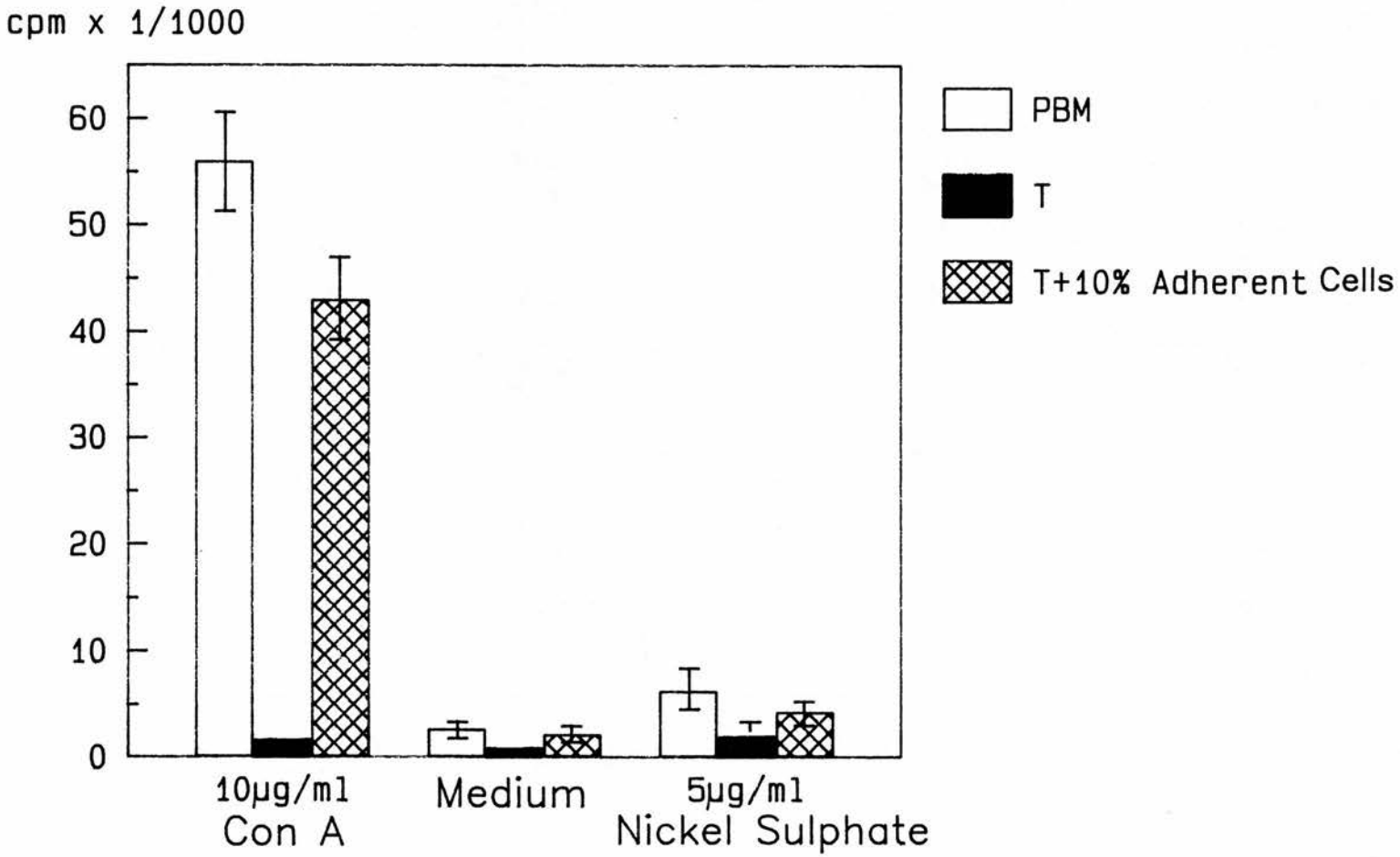


Stimulation of PBM, nylon wool enriched T-cells, and nylon wool enriched T-cells with added irradiated plastic-adherent blood cells (10%) from a nickel-sensitive patient using medium, 10µg/ml Con A and 5µg/ml $NiSO_4$ in a 6 day LTT. Results in cpm x 10^{-3} (Mean ± SD).

Fig 4.5

Reconstitution of T-Cell Response by Plastic-Adherent Blood Cells

Control 13a



Stimulation of PBM, nylon wool enriched T-cells, and nylon wool enriched T-cells with added irradiated plastic-adherent blood cells (10%) from a non-sensitised control using medium, 10µg/ml Con A and 5µg/ml $NiSO_4$ in a 6 day LTT. Results in cpm x 10^{-3} (Mean ± SD).

The Accessory Cell Function Of Epidermal Cells

Having established a method to investigate the abilities of plastic-adherent blood cells to act as accessory cells, the accessory cell function of epidermal cells was investigated. In these experiments, only a limited number of epidermal cells could be obtained. Therefore, the 6 day LTT was carried out with $2x10^5$ PBM/well and also 10^5 PBM/well to determine whether fewer lymphocytes could be used effectively in the assay.

Results from one experiment using cells from a nickel-sensitive patient (P16) and a non-sensitised control (C24) are shown in fig 4.6 and Table 4.4. When 10^5 PBM/well were incubated with either 5µg/ml or 10µg/ml of nickel sulphate, the SI obtained decreased and the patient was not positive in the assay whereas positive SIs were obtained using $2x10^5$ cells/well. Therefore, $2x10^5$ cells/well were used in the epidermal cell reconstitution experiments.

Table 4.4 Stimulation Indices Obtained using $2x10^5$ and 10^5 PBM/well in the 6 Day LTT

	$2x10^5$ cells/well		10^5 cells/well	
	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$
P16	3.5	4.0	2.5	2.7
C24	1.5	1.3	1.3	1.0

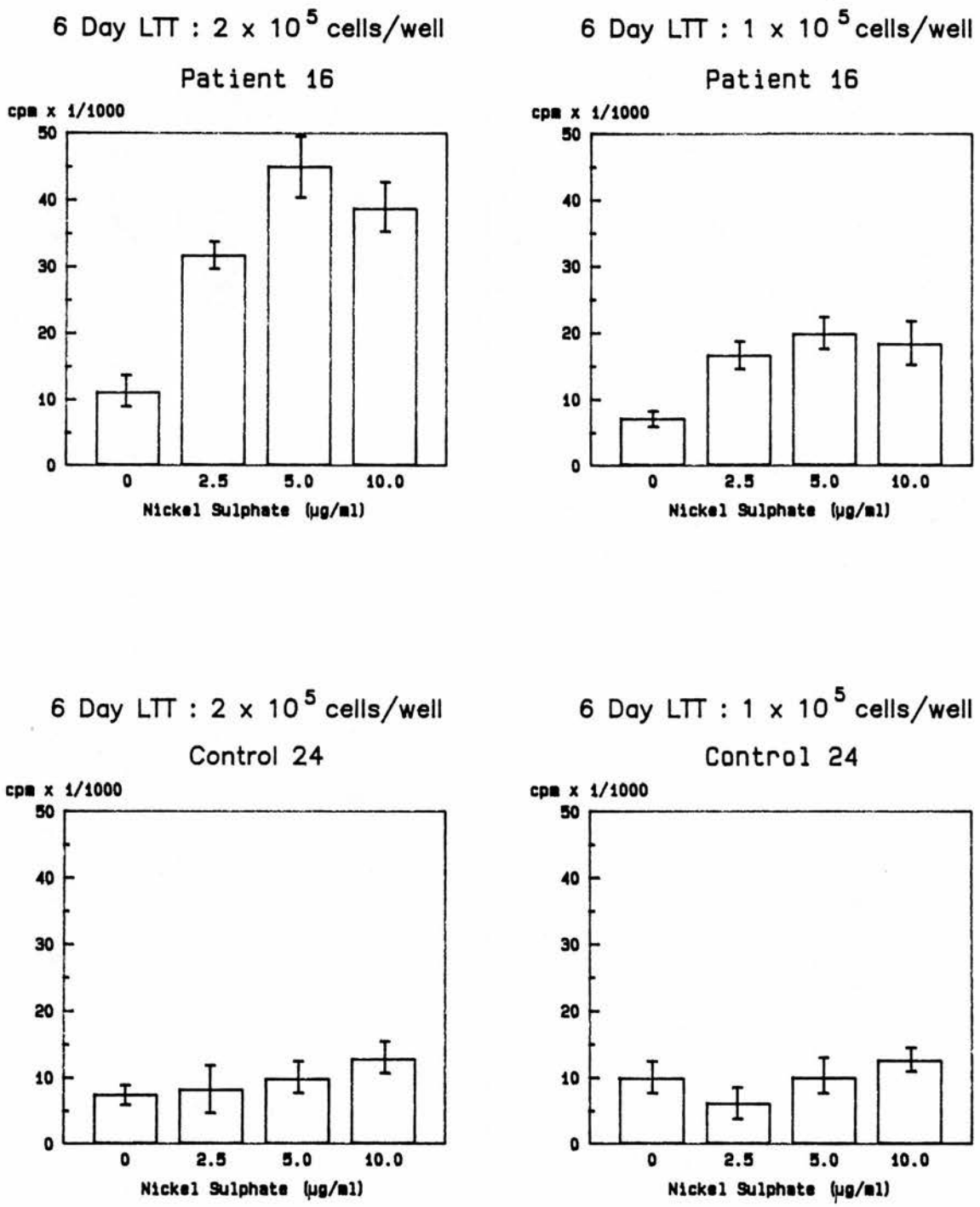


Fig 4.6

To establish whether $1x10^5$ cells/well could be used successfully in the LTT, $1x10^5$ PBM/well and $2x10^5$ PBM/well (normally used in the experiments described in this thesis) from 1 nickel-sensitive patient and 1 non-sensitised control were incubated with 0-10µg/ml $NiSO_4$ in the 6 day LTT. Results in cpm x 10^{-3} (Mean ± SD).

The Ability of Epidermal Cells to Act as Accessory Cells in the Presence of Con A

Studies carried out by Braathen and Thorsby (1980) indicated that epidermal accessory cell function required a T-cell:epidermal cell ratio of 10:4 (i.e. 40% epidermal cells). Therefore, up to 40% epidermal cells were used in the following experiments to determine their accessory cell function. Initial experiments were carried out using cells from control subjects in a 5 day assay with Con A. As previously described, nylon wool enriched T-cells (10^6) were incubated with 10µg/ml of Con A in the presence or absence of either 10^5 (10%) plastic-adherent blood cells or with 10^5-$4x10^5$ (10-40%) epidermal cells and the results compared with those obtained using PBM. The stimulation indices obtained using nylon wool enriched T-cells with or without accessory cells and PBM are shown in Table 4.5.

Table 4.5

Stimulation Indices Obtained Using 10^6 PBM or 10% Plastic-Adherent Blood Cells and 10-40% Epidermal Cells With 10^6 Nylon Wool Enriched T-Cells

Expt	PBM	T	T+10%Ad	T+10%Ep	T+20%Ep	T+40%Ep
E1	142.0	1.0	23.3			91.5
E2	65.0	2.0	30.0			44.0
E3	18.0	3.0	26.0	6.4	6.0	18.0
E4	10.0	2.0	5.4	4.2	5.0	4.2

The addition of 40% epidermal cells to the T-cells gave a good reconstitution of the Con A response in the first three experiments. Reconstitution in the final experiment was similar when three epidermal cell concentrations (10%, 20% and 40%) were used. Experiments were then carried out using 40% (and if possible, 20%) epidermal cells to assess their ability to present the antigen nickel sulphate to enriched T-cells.

The Ability of Epidermal Cells to Act as Accessory Cells in the Presence of Nickel Sulphate

The ability of epidermal cells to present both Con A and nickel sulphate to nylon wool enriched T-cells was investigated using cells obtained from 5 patients and 4 controls in a 6 day LTT. Experimental results are shown in Appendices VII and VIII (pages 190-91). Stimulation indices obtained using mitogen and 5µg/ml nickel sulphate are shown in Table 4.6. Results from one patient (P48) and one control (C31) are displayed graphically in figs 4.7 and 4.8.

Addition of both 20% and 40% epidermal cells to the enriched T-cells reconstituted or enhanced the Con A response in all patients and controls (Table 4.6, columns 5 and 6) and increased the mitogenic response of highly enriched T-cells from P48 and C31 in relation to the stimulation of PBM (see figs 4.7 and 4.8). A T-cell response against nickel sulphate was evident only when cells from nickel-sensitive patients were used (Table 4.6. columns 7-11; figs 4.7 and 4.8).

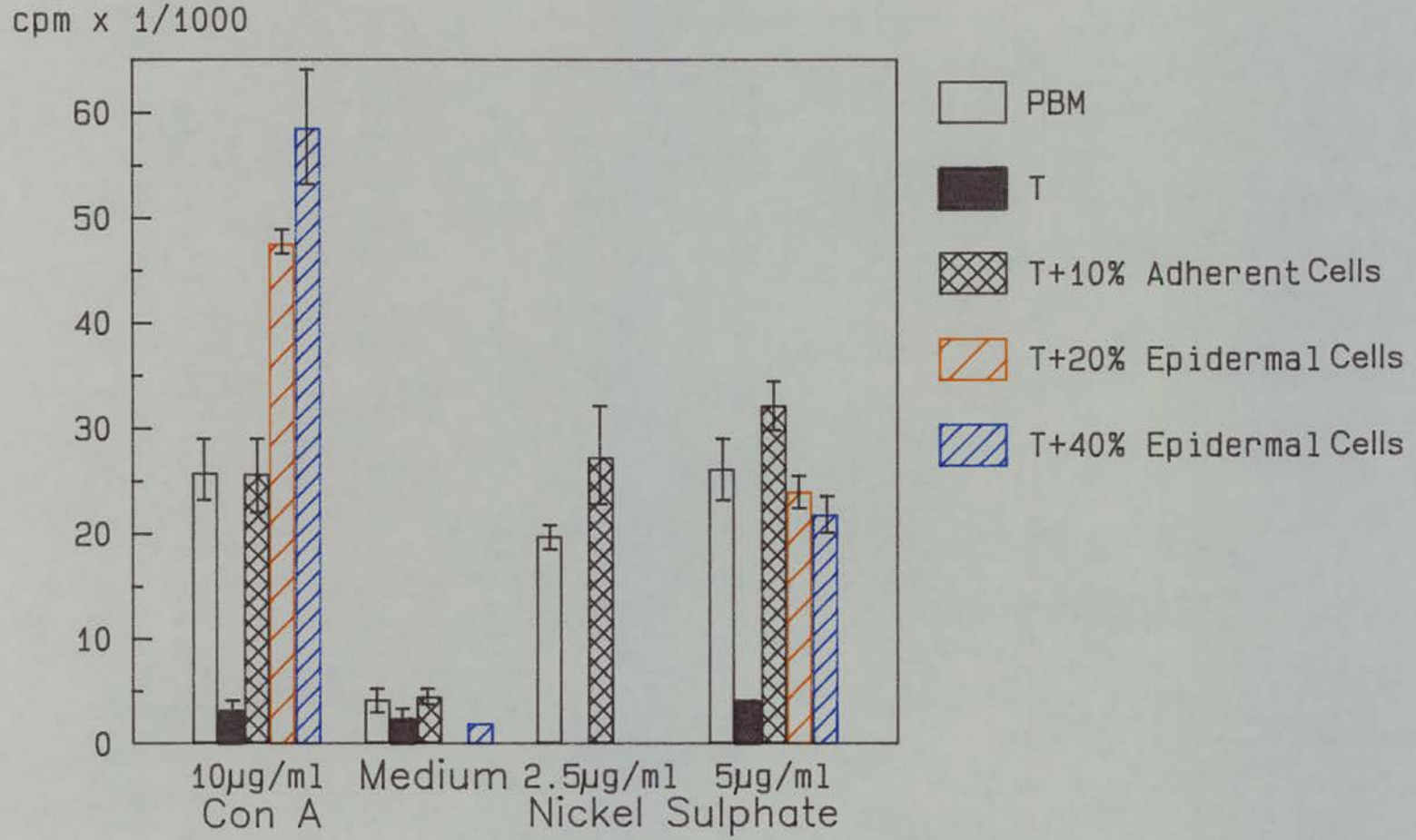
The T-cell responses in the presence of both 20% and 40% epidermal cells were not significantly different from those obtained using PBM. The epidermal cells contained approximately 2% MHC II^+ cells in contrast to 40-50% MHC II^+ cells in the population of plastic-adherent blood cells. The experimental finding that 20% epidermal cells are more effective than 10% plastic-adherent blood cells in permitting mitogen- or antigen-induced T-cell proliferation supports the statement by Braathen and Thorsby (1983) that epidermal accessory cells are more efficient than blood accessory cells on a per cell basis.

Table 4.6 Stimulation Indices Obtained using Con A and 5µg/ml Nickel Sulphate with PBM and Enriched T-Cells with or without Added Plastic-Adherent Blood Cells or Epidermal Cells

	10µg/ml Con A					5µg/ml $NiSO_4$				
Pat	PBM	T	T+10%Ad	T+20%Ep	T+40%Ep	PBM	T	T+10%Ad	T+20%Ep	T+40%Ep
P20	4.4	1.6	3.6	3.0	5.2	8.9	3.8	12.3	11.9	11.5
P41	3.3	1.3	2.1	5.6	7.8	3.2	N.T.	3.6	5.6	6.5
P46	3.5	1.6	8.4	22.7	28.3	3.8	0.9	3.7	5.9	6.9
P48	6.1	1.3	5.7	23.8	29.3	6.2	1.7	6.2	12.0	11.0
P62	28.8	14.0	42.9	72.2	102.0	9.1	10.1	13.7	23.0	28.4
Cont										
C11	9.0	2.5	8.1	15.2	8.6	2.8	1.3	1.6	2.6	2.4
C12	19.3	1.5	13.2	11.6	11.0	1.4	1.0	1.6	N.T.	1.5
C26	9.9	1.1	9.8	14.4	28.3	2.0	0.8	2.3	1.8	2.8
C31	18.3	1.7	30.0	42.2	72.5	0.4	1.7	1.3	1.0	0.5

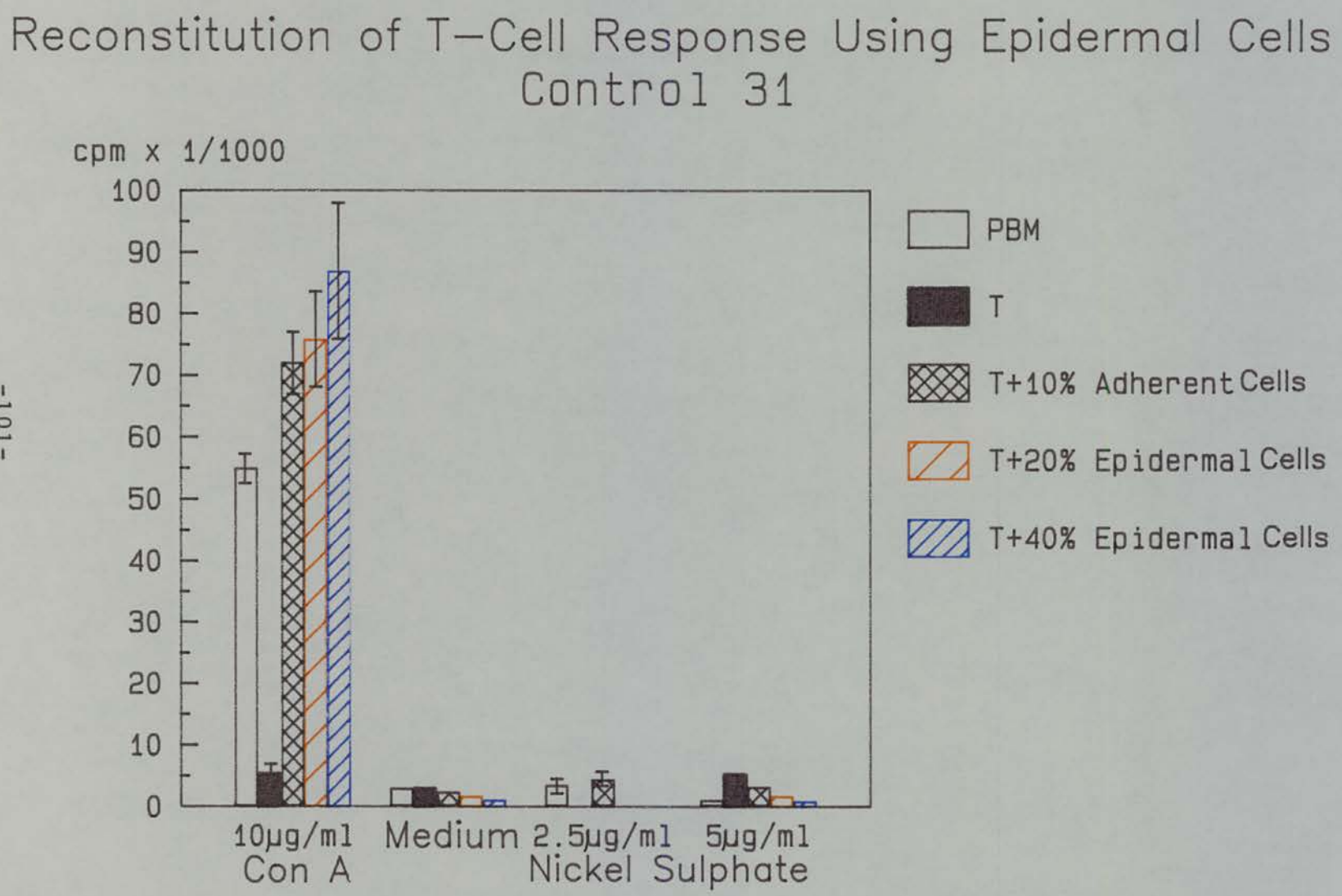
Fig 4.7

Reconstitution of T-Cell Response Using Epidermal Cells
Patient 48



Stimulation of PBM, nylon wool enriched T-cells, and nylon wool enriched T-cells with added irradiated plastic-adherent blood cells (10%) and epidermal cells (20% and 40%) from a nickel-sensitive patient using medium, 10µg/ml Con A and 2.5µg/ml and 5µg/ml $NiSO_4$ in a 6 day LTT. Results in cpm x 10^{-3} (Mean ± SD).

Fig 4.8



Stimulation of PBM, nylon wool enriched T-cells, and nylon wool enriched T-cells with added irradiated plastic-adherent blood cells (10%) and epidermal cells (20% and 40%) from a non-sensitised control using medium, 10µg/ml Con A and 2.5µg/ml and 5µg/ml $NiSO_4$ in a 6 day LTT. Results in cpm x 10^{-3} (Mean ± SD).

THE ROLE OF MHC II ANTIGENS (HLA-DP, -DQ, -DR) IN T-CELL PROLIFERATION

It is now well established that antigen-specific T-cell proliferation requires the presence of antigen presenting cells expressing MHC II molecules. At present, the human class II MHC is known to contain three regions encoding for HLA-DP, -DQ and -DR molecules. Studies to assess the role of these three different molecules can be carried out using antibodies directed against particular class II MHC antigens.

Three methods can be employed to determine the effects of antibodies against cell surface antigens on cell proliferation (Akiyama et al., 1985).

(1) Lysis of MHC II^+ cells using specific antibodies and complement.

(2) Pulsing the cells with antibody.

(3) Maintaining the antibody in the culture.

In the following chapter, the role of class II antigens in permitting cell proliferation was studied using 5 monoclonal antibodies (details shown on page 61):-

DA6.231	Anti-HLA-DP, -DQ and -DR
L243	Anti-HLA-DR
B7/21	Anti-HLA-DP
Leu 10	Anti-HLA-DQ
L368	Anti-Beta$_2$microglobulin (used as a negative control).

With the exception of antibody DA6.231, the antibodies used were in limited supply. Therefore, a method was needed which allowed the lowest possible concentrations of antibody to be used.

(1) Destruction of MHC II$^+$ Cells Using Antibody DA6.231 And Complement

The requirement for HLA-DR$^+$ cells for T-cell antigen- or mitogen-induced proliferation is well documented. Therefore, this proliferation should be inhibited if antibodies against HLA-DR are used successfully. In order to show that the antibodies were having a specific inhibitory effect, it was hoped to obtain 50% inhibition of the antigen-specific proliferative response.

The destruction of cells expressing MHC II antigens (including HLA-DR) was attempted using antibody DA6.231 and complement (C'). Like most monoclonal antibodies, DA6.231 is an IgG antibody (subclass IgG1) and consequently cannot fix complement directly. Therefore, PBM were incubated with DA6.231, then rabbit anti-mouse serum, and finally complement (See Materials and Methods, page 61).

Stimulation indices obtained for the populations of cells undergoing different treatments with the antibodies and complement are shown in Table 5.1, together with the percentage inhibition of the Con A response obtained using untreated cells.

Table 5.1 Inhibition of the Con A Response by Destroying MHC II$^+$ Cells with DA6.231 and Complement

	+ Reagent			SI	% Inhib
	DA6.231	Rb anti-mIg	C'		
(a)	-	-	-	4.8	0
(b)	-	-	+	3.3	31
(c)	-	+	-	4.4	8
(d)	-	+	+	3.2	33
(e)	+	-	-	3.5	27
(f)	+	+	-	3.1	35
(g)	+	-	+	4.6	4
(h)	+	+	+	3.2	33

As shown in Table 5.1, this method did not specifically inhibit the Con A response; inhibition of the Con A response after incubation of the cells with the two antibodies and complement (h) did not differ from some of the inhibitions obtained using cells incubated with only one (b) or two (d and f) of the three reagents required.

(2) Inhibition of Cell Proliferation by Pulsing Cells with Monoclonal Antibodies

Experiments were then carried out to assess the inhibitory effects of antibodies against MHC II molecules using the method of pulsing. PBM were incubated with the antibodies for one hour and were then washed (to remove excess antibody) before being placed in culture.

Results obtained using cells from 2 nickel-sensitive patients (P42 and P43) and 1 nickel non-sensitive control (C12) pulsed with DA6.231 at very high concentrations and then stimulated by Con A and nickel sulphate are shown in figs 5.1a and 5.1b. Inhibition of the antigen- and mitogen-induced response was evident using cells from one patient (P43). However, there was no inhibition of the response of cells taken from the other patient (P42), even when 3.9µg/ml (3900ng/ml) of DA6.231 was used.

Inhibition of Con A–Induced Cell Proliferation

Pulsing (Antibody DA6.231)

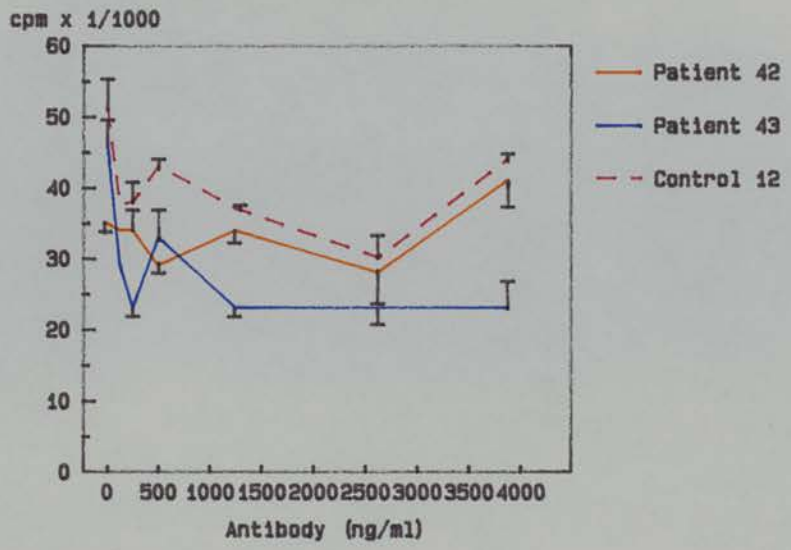


Fig 5.1a

Inhibition of Nickel Sulphate–Induced Cell Proliferation

Pulsing (Antibody DA6.231)

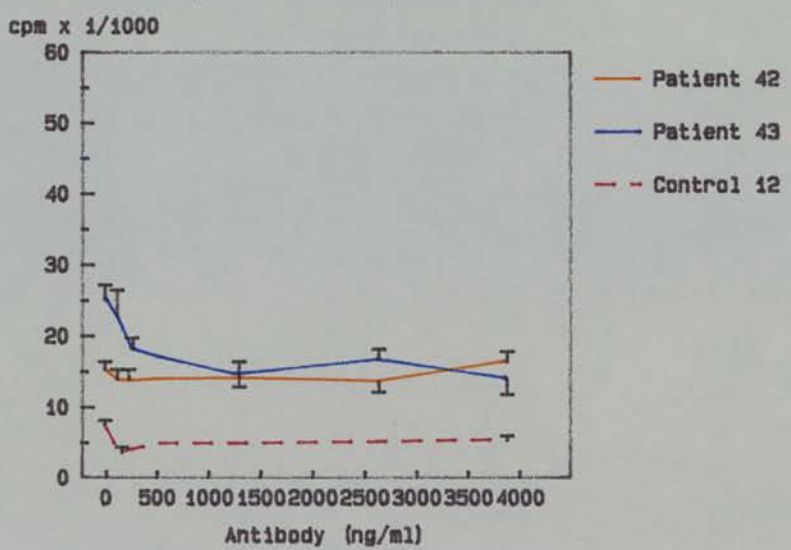


Fig 5.1b

PBM were incubated with 0-4µg/ml of antibody DA6.231 for 1 hour at room temperature. The cells were washed 3 times with PBS and then incubated with medium, 10µg/ml Con A (fig 5.1a) or 5µg/ml $NiSO_4$ (fig 5.1b) in the 6 day LTT. Results in cpm x 10^{-3} (Mean ± SD).

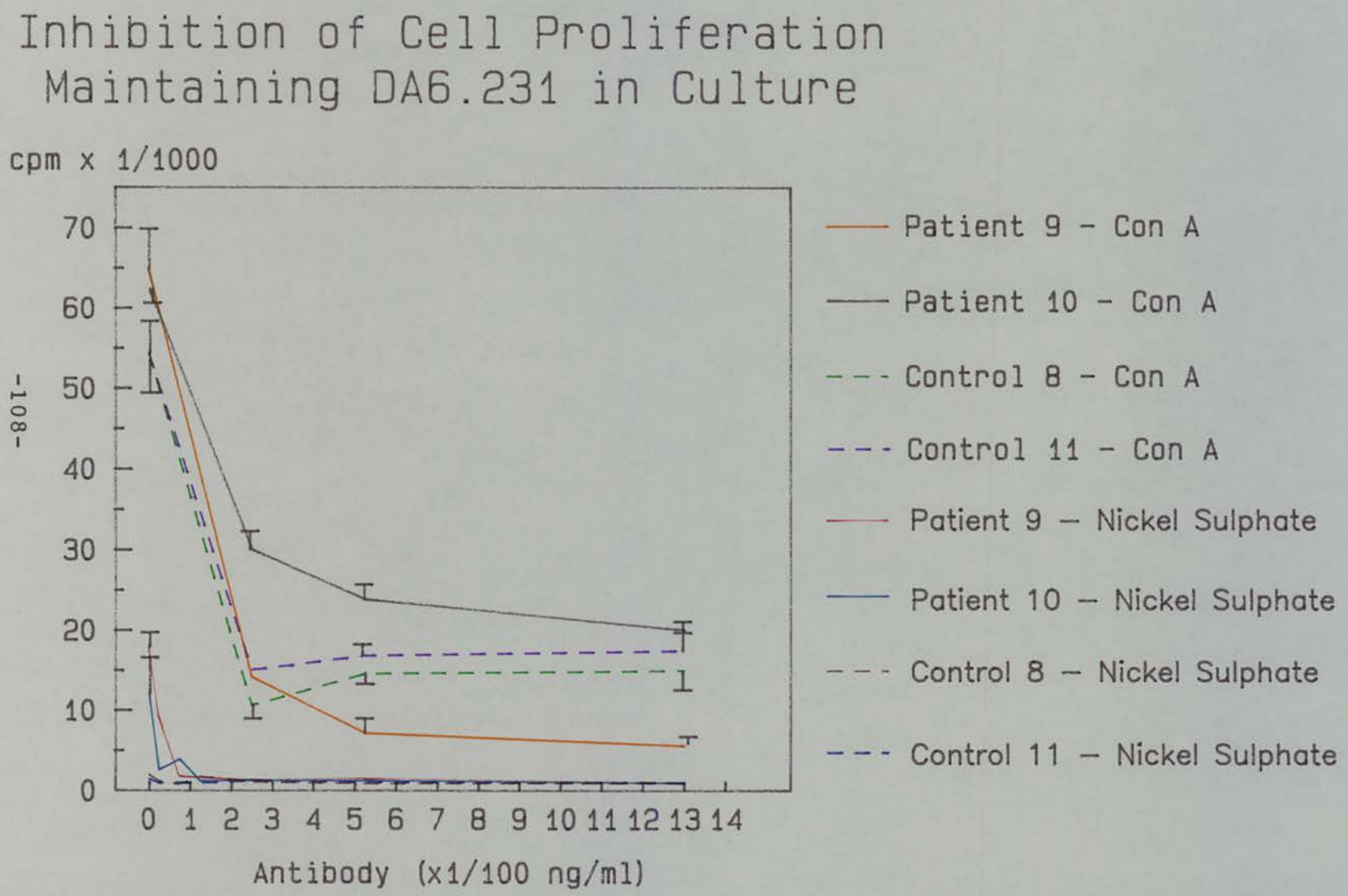
Results obtained when pulsing PBM with different concentrations of 4 antibodies (DA6.231, B7/21, Leu 10 and L368) are shown in Appendices IX.1-IX.8 (pages 192-96). At the lower antibody concentrations studied, there were no alterations in the response of cells taken from a nickel-sensitive patient (P38) against either Con A or nickel sulphate. However, the Con A response of cells taken from a control individual (C17) was inhibited (45% inhibition) when 2.6μg/ml of DA6.231 was used.

When the higher concentrations of antibody were used (Appendices X.1-X.8, pages 197-201), the Con A responses of cells from both the nickel-sensitive patient (P11) and the control (C11) were not inhibited (inhibition < 45%) by any of the antibodies. However, the antigen-specific response of the patient's cells was inhibited by 50% by all of the antibodies when used at concentrations of 2μg/ml, including L368 which is an antibody directed against MHC I antigens and which was not expected to affect cell proliferation. Therefore, because of this inhibition by the control antibody at high concentrations and because of antibody availability, experiments were carried out to study the effects on antigen- and mitogen-induced cell proliferation of maintaining DA6.231 in culture.

(3) Inhibition of Cell Proliferation by Maintaining Antibody DA6.231 in Culture

Initial studies were performed to determine the ability of DA6.231 to inhibit antigen- and mitogen-induced cell proliferation when the antibody was maintained in the culture system throughout the 6 day incubation of the LTT. Fig 5.2 shows the results obtained using cells from two nickel-sensitive patients (P9 and P10) and two non-sensitised controls (C8 and C11). Antibody concentrations greater than 130ng/ml ablated the antigen-specific responses. These concentrations also permitted some cell proliferation in the presence of Con A, which probably indicated that the antibodies were not directly cytotoxic to the cells.

Fig 5.2



PBM from 4 nickel-sensitive patients and 4 non-sensitised controls were incubated with 0-1400ng/ml DA6.231 for 1 hour at room temperature. 10µg/ml Con A or 5µg/ml $NiSO_4$ were then added to the cells and the 6 day LTT carried out. Results in cpm x 10^{-3} (Mean ± SD).

A Comparison of the Effects on Cell Proliferation of Pulsing Cells with Antibody and Maintaining the Antibody in the Culture

To compare the effects on cell proliferation of pulsing cells with antibody and maintaining the antibody in the culture system, PBM from 3 nickel-sensitive patients (P28, P29 and P34) and 1 control (C21) were either pulsed with the monoclonal antibody DA6.231 at varying concentrations before the addition of 5μg/ml of nickel sulphate or were incubated with the antibody and 5μg/ml of nickel sulphate for 6 days in the LTT.

The results obtained are shown in figs 5.3a-5.3d. In these experiments, PBM from all 3 nickel-sensitive patients pulsed with the antibody at the higher concentrations (> 520ng/ml) showed more than 50% inhibition of the antigen-specific cell proliferation. However, the use of 130-260ng/ml of antibody maintained within the culture markedly inhibited the antigen-specific response (80% inhibition). In addition, the viability of the cells after culture with the antibody for 6 days was determined using trypan blue exclusion. Cells were more than 90% viable, indicating that the antibody was not preventing cell proliferation because of cytotoxicity. An antibody concentration of 260ng/ml was therefore used in a series of experiments designed to investigate the effects of monoclonal antibodies against different MHC molecules on accessory cell function.

Fig 5.3

Pulsing

PBM from 3 nickel-sensitive patients and 1 non-sensitised control were incubated with 0-2.6μg/ml of DA6.231 for 1 hour at room temperature and then washed 3 times before 10μg/ml Con A and 5μg/ml $NiSO_4$ were added to the cells and the 6 day LTT was carried out.

In Culture

The results obtained were compared with those obtained using PBM from the same patients and control incubated with 10μg/ml Con A or 5μg/ml $NiSO_4$ and 0-520ng/ml DA6.231 throughout the 6 day LTT.

Results in cpm x 10^{-3} (Mean $\pm$ SD).

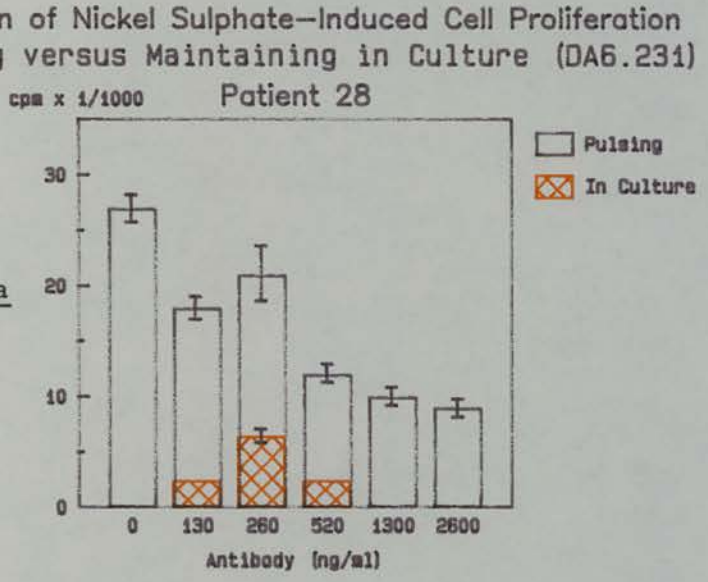


Fig 5.3a

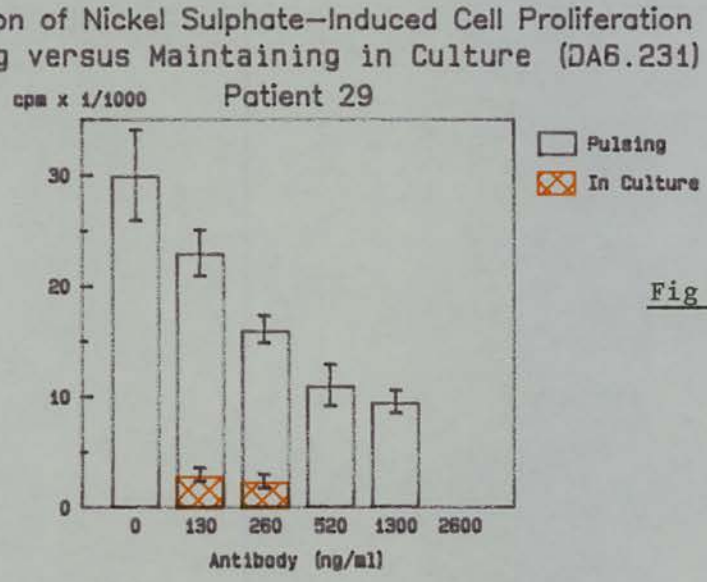


Fig 5.3b

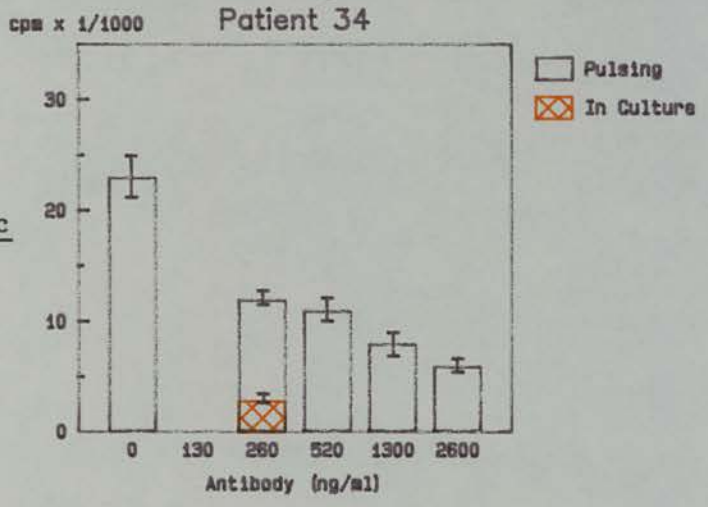


Fig 5.3c

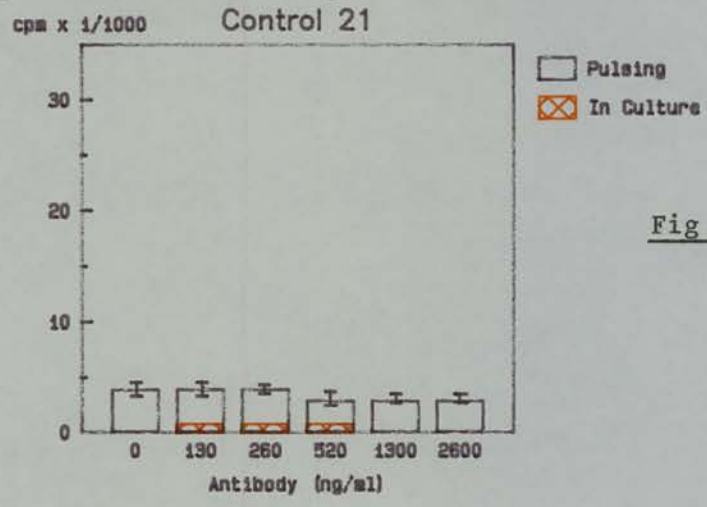


Fig 5.3d

Inhibition of Cell Proliferation by Antibodies Results from the Inhibition of Accessory Cell Function

Additional experiments were carried out to ensure that the antibodies against HLA-DR were not inhibiting cell proliferation either because of cytotoxicity, or by directly preventing either the proliferation of activated T-cells or the cellular incorporation of tritiated thymidine. Accessory cell function takes place within the first 24 hours of culture. Therefore, if the antibody inhibition is specifically due to the prevention of accessory cell function, cell proliferation should not be affected by the addition of antibody to the cells after a few days of culture, when successful mitogen- or antigen-induced cell activation should already have taken place.

As stated previously (page 107), the Con A response was not totally inhibited by the addition of 260ng/ml of DA6.231. As many of the cells in culture are capable of responding to 10μg/ml of Con A, it was possible that the anti-HLA-DR antibody may have been sufficiently cytotoxic to prevent antigen-induced cell proliferation, whilst not inhibiting totally the mitogen-induced response. In the following experiments, this was investigated using cells from 3 patients (P17, P20 and P55) and 3 controls (C28, C30 and C41). The cells were divided into three groups:-

(a) No antibody in the culture
(b) Antibody added to the culture on day 0
(c) Antibody added to the culture on day 3

and the 6 day LTT was carried out. The results obtained using antibodies DA6.231, L243 and L368 are shown in figs 5.4 to 5.6.

DA6.231 (figs 5.4a-5.4f)

1µg/ml of Con A

Maintenance of DA6.231 in the culture for 6 days inhibited the response of cells from all 3 nickel-sensitive patients and non-sensitised controls against 1µg/ml of Con A; the response was not affected by the addition of antibody on day 3 in all subjects except Patient 55 (fig 5.4b).

10µg/ml of Con A

The response to 10µg/ml of Con A was inhibited when cells from controls C28, C30 and C41 were cultured in the presence of DA6.231 for 6 days but not when the antibody was added to the cultures on the third day of incubation (except for C30, Fig 5.4d). Cells from Patient 55 incubated with 10µg/ml of Con A were not inhibited by the maintenance of DA6.231 in culture (fig 5.4b).

Medium

Addition of DA6.231 for the 6 days of culture inhibited the background proliferation of cells from controls C28 and C41.

5µg/ml of Nickel Sulphate

Addition of DA6.231 for the 6 days of culture significantly inhibited (> 50%) the antigen-specific response of the cells from all three patients, and this inhibition was absent when antibody was added to the cells on the third day of culture.

L368 (figs 5.5a-5.5b)

Addition of the control antibody L368 for the whole of the 6 day culture inhibited the response of cells from control C28 against 10µg/ml of Con A. There was no inhibition of cell proliferation in the presence of other stimulants, or when the antibody was added to the cells on the third day of culture.

L243 (figs 5.6a-5.6d)

1µg/ml of Con A

The maintenance of antibody L243 during the 6 days of culture inhibited the response against 1µg/ml of Con A of PBM from the 4 subjects studied. There was partial inhibition of the response of cells from Control 41 following the addition of L243 on the third day of culture (fig 5.6c).

10µg/ml of Con A

Maintenance of L243 in the 6 day culture did not inhibit the responses of cells from Patient 55 against 10µg/ml of Con A (fig 5.6b). Proliferation of the cells from the remaining three subjects was inhibited (50%) but there was no effect when the antibody was added on the third day of culture.

Medium

Addition of L243 decreased the background proliferation of the cells from Control 41.

5µg/ml of Nickel Sulphate

Maintenance of L243 in the culture for 6 days inhibited the antigen-specific response of cells from nickel-sensitive patients P20 and P55 (figs 5.4a,b); addition of the antibody on day three of the culture permitted antigen-induced cell proliferation.

The results of these experiments indicated that the inhibition seen when 260ng/ml of either DA6.231 or L243 is added to PBM in the presence of mitogen or antigen and left in the culture for the 6 day period of the LTT, results from an inhibition of accessory cell function, and not from either antibody cytotoxicity or the direct prevention of the cellular incorporation of tritiated thymidine.

Figs 5.4-5.6

Figs 5.4a-5.4f

PBM from 3 nickel-sensitive patients and 3 non-sensitised controls were incubated with 1 and 10μg/ml Con A, medium and 5μg/ml $NiSO_4$ in the 6 day LTT. To assess whether DA6.231 was inhibiting cell proliferation after T-cell activation, 260ng/ml antibody was added to the cells before culture (Antibody Day 0) and when the cells had been incubated for 2 days (Antibody Day 3). Results in cpm x 10^{-3} (Mean ± SD).

Figs 5.5a-5.5b

PBM from 1 nickel-sensitive patient and 1 non-sensitised control were incubated with 1 and 10μg/ml Con A, medium and 5μg/ml $NiSO_4$ in the 6 day LTT. To assess whether L368 could affect cell proliferation after T-cell activation, 260ng/ml antibody was added to the cells before culture (Antibody Day 0) and when the cells had been incubated for 2 days (Antibody Day 3). Results in cpm x 10^{-3} (Mean ± SD).

Figs 5.6a-5.6d

PBM from 2 nickel-sensitive patients and 2 non-sensitised controls were incubated with 1 and 10μg/ml Con A, medium and 5μg/ml $NiSO_4$ in the 6 day LTT. To assess whether L243 was inhibiting cell proliferation after T-cell activation, 260ng/ml antibody was added to the cells before culture (Antibody Day 0) and when the cells had been incubated for 2 days (Antibody Day 3). Results in cpm x 10^{-3} (Mean ± SD).

Specificity of Antibody Inhibition
Antibody DA6.231 (260ng/ml) Patient 20

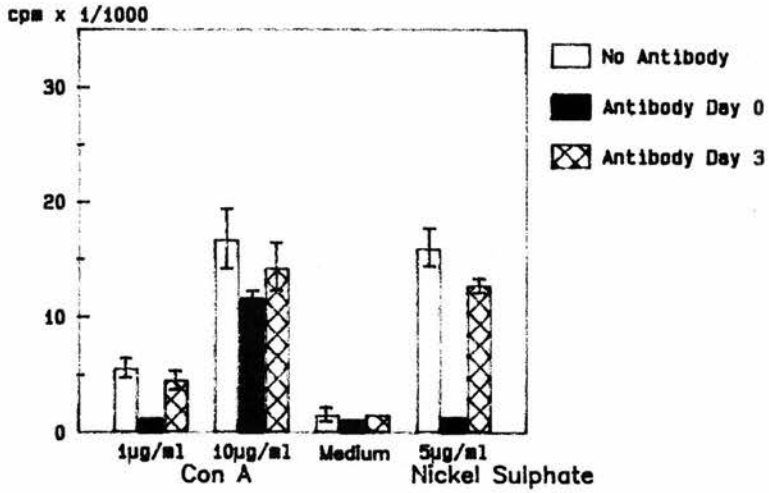


Fig 5.4a

Specificity of Antibody Inhibition
Antibody DA6.231 (260 ng/ml) Patient 55

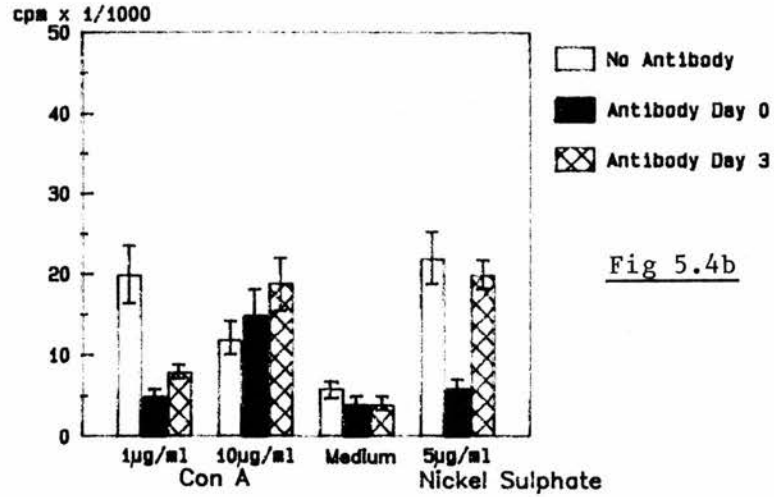


Fig 5.4b

Specificity of Antibody Inhibition
Antibody DA6.231 (260ng/ml) Control 41

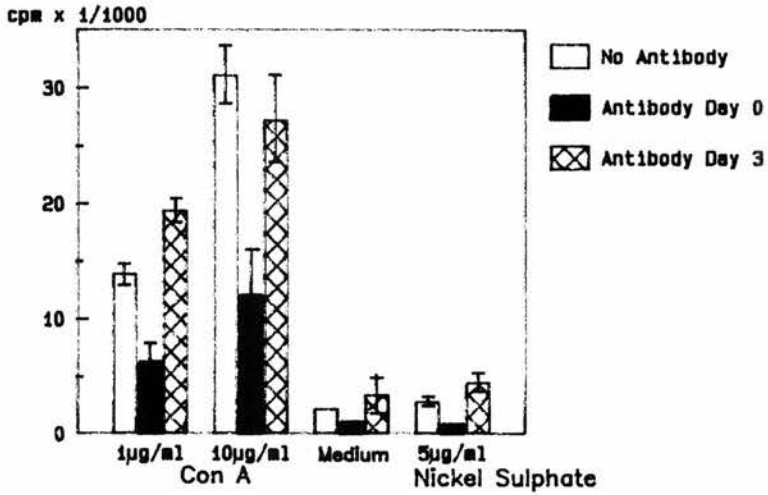


Fig 5.4c

Specificity of Antibody Inhibition
Antibody DA6.231 (260 ng/ml) Control 30

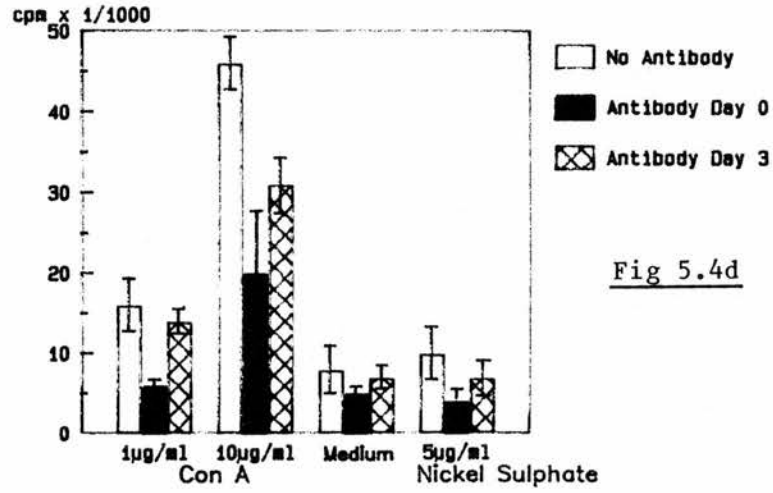


Fig 5.4d

Specificity of Antibody Inhibition
Antibody DA6.231 (260ng/ml) Patient 17

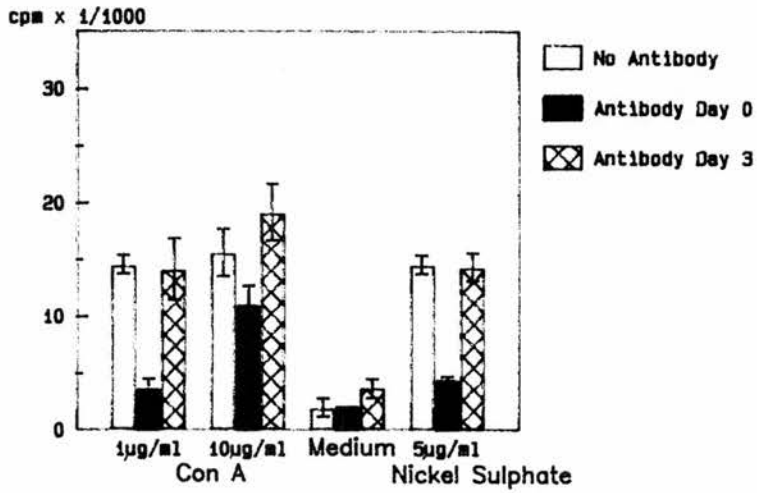


Fig 5.4e

Specificity of Antibody Inhibition
Antibody L368 (260ng/ml) Patient 17

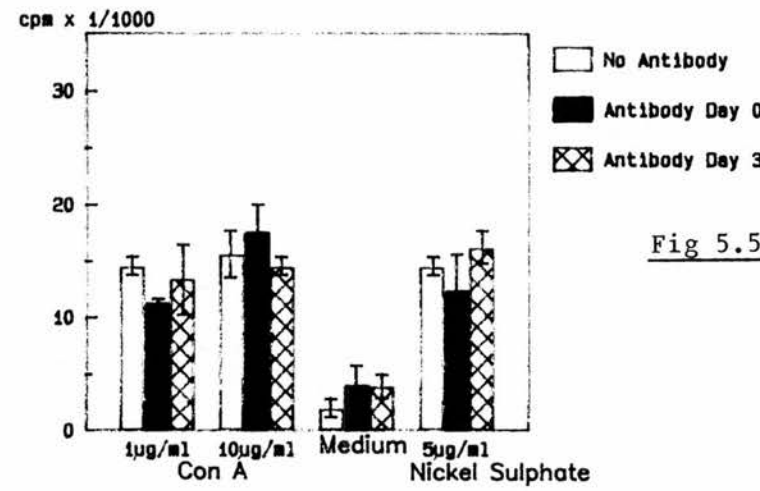


Fig 5.5a

Specificity of Antibody Inhibition
Antibody DA6.231 (260ng/ml) Control 28

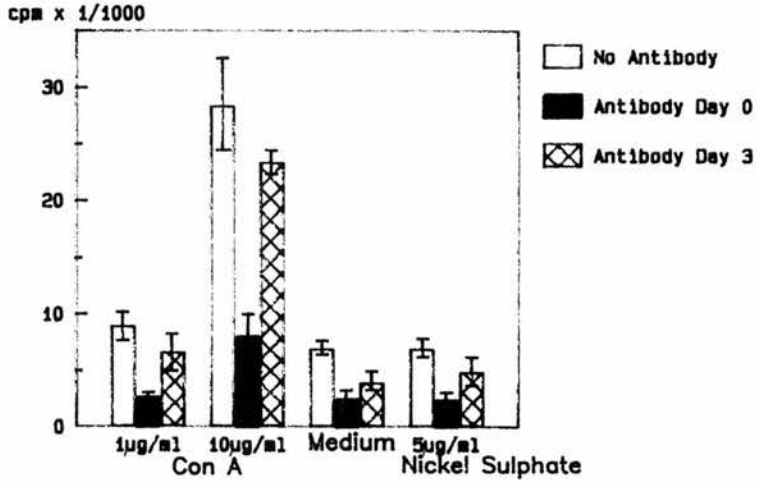


Fig 5.4f

Specificity of Antibody Inhibition
Antibody L368 (260ng/ml) Control 28

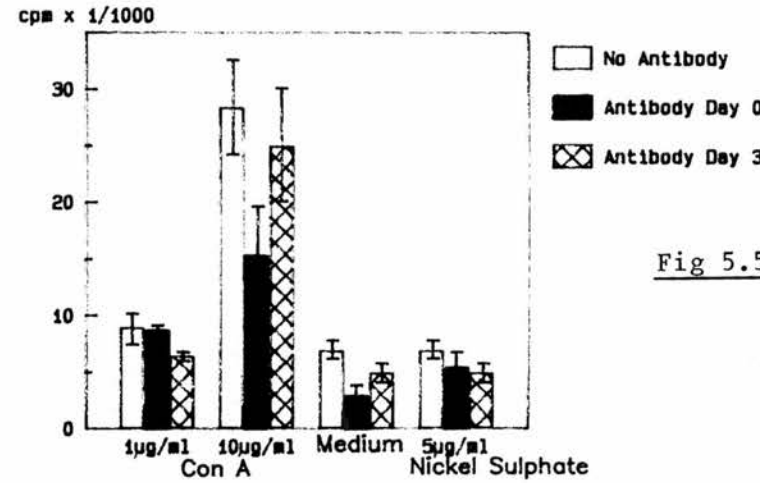


Fig 5.5b

Specificity of Antibody Inhibition
Antibody L243 (260ng/ml) Patient 20

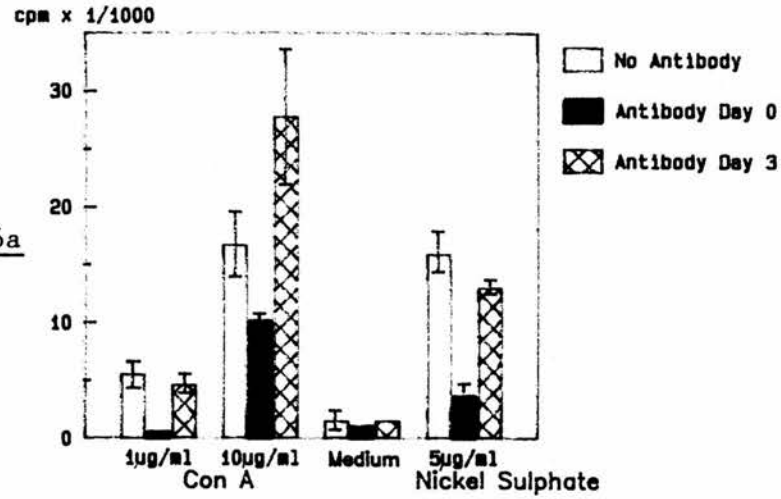


Fig 5.6a

Specificity of Antibody Inhibition
Antibody L243 (260 ng/ml) Patient 55

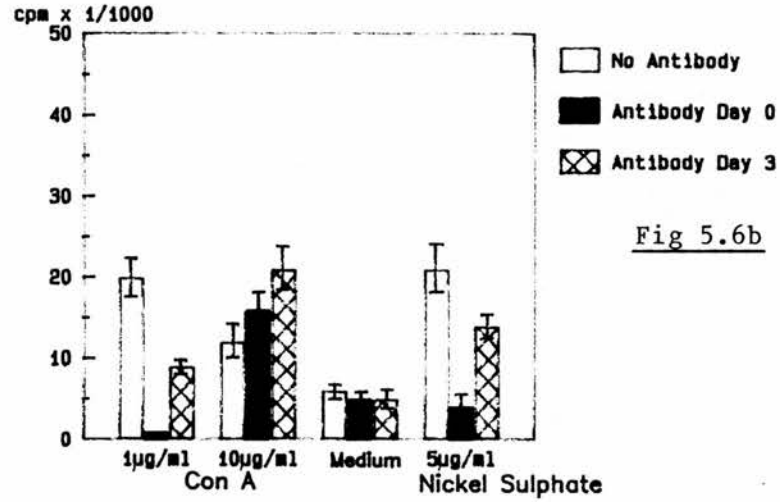


Fig 5.6b

Specificity of Antibody Inhibition
Antibody L243 (260ng/ml) Control 41

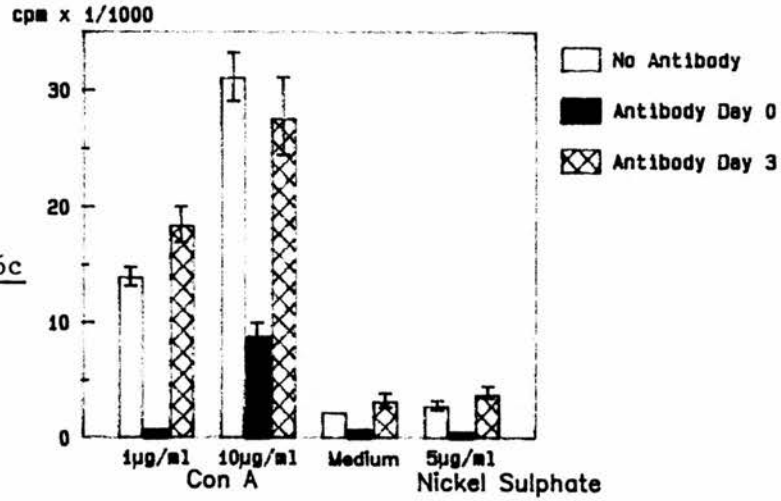


Fig 5.6c

Specificity of Antibody Inhibition
Antibody L243 (260 ng/ml) Control 30

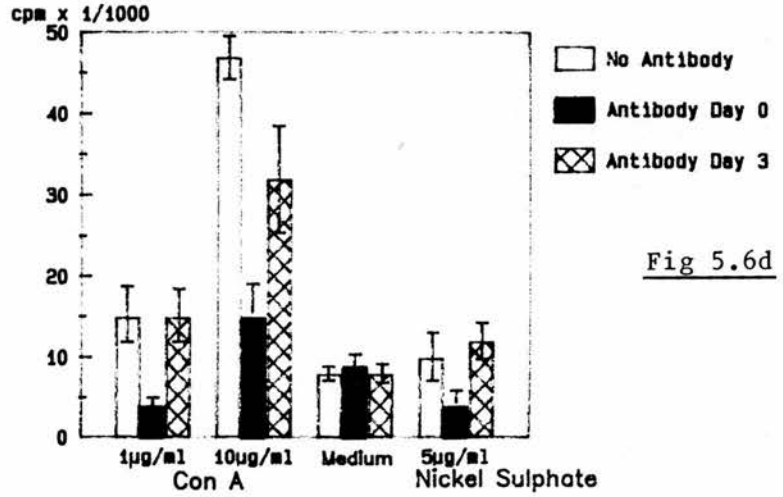


Fig 5.6d

The Role of Different MHC Molecules in PBM Proliferation

The effects on PBM proliferation of five antibodies (DA6.231, L243, B7/21, Leu 10 and L368) left in the culture in a 6 day LTT were determined using 0-260ng/ml of antibody. PBM were incubated with antibody for one hour before either 10μg/ml of Con A or 5μg/ml of nickel sulphate was added and the 6 day LTT carried out.

The radioactive counts obtained in these experiments are shown in Appendix XI (Figs XI.1-XI.5, pages 202-6). The percentage inhibitions of cell proliferation obtained using each of the five antibodies are shown in figs 5.7-5.11. The ranges of percentage inhibition of cell proliferation obtained using 260ng/ml of antibody are shown in Table 5.2.

Table 5.2 Inhibition of Cell Proliferation Obtained using 260ng/ml of Antibody

Antibody	MHC Antigen	% Inhib 10μg/ml Con A	% Inhib 5μg/ml $NiSO_4$
DA6.231	HLA-DP, -DQ, DR	10, +51 - +85	+82 - +93
L243	HLA-DR	17, +40 - +98	+87 - +96
B7/21	HLA-DP	-35 - +42	+32 - +58
Leu 10	HLA-DQ	-63 - +55	-65 - +36
L368	$Beta_2$microglobulin	-52 - +21	00 - +27

(-% = stimulation)

The inhibition of antigen- and mitogen-induced PBM proliferation using antibodies DA6.231 and L243 against HLA-DR was dose dependent (figs 5.7 and 5.8); nickel sulphate-induced cell proliferation was inhibited by more than 82% by 260ng/ml of antibody. The results obtained using the remaining three antibodies were more variable; B7/21 (anti-HLA-DP, fig 5.9) inhibited the antigen-specific response (32-58% inhibition) but did

not affect mitogen-induced proliferation. The addition of antibody Leu 10 (anti-HLA-DQ, fig 5.10) had variable effects on mitogen- and antigen- induced PBM proliferation, producing both inhibition and stimulation. Finally, the control antibody L368 (anti-beta$_2$-microglobulin, figs 5.11), had variable effects on the mitogen-induced proliferation and weakly inhibited (0-27%) the antigen-specific response.

These experiments indicate that HLA-DR molecules are important in permitting antigen-specific PBM proliferation and suggest that the expression of HLA-DP molecules may also be required for antigen-specific T-cell activation.

Fig 5.7a

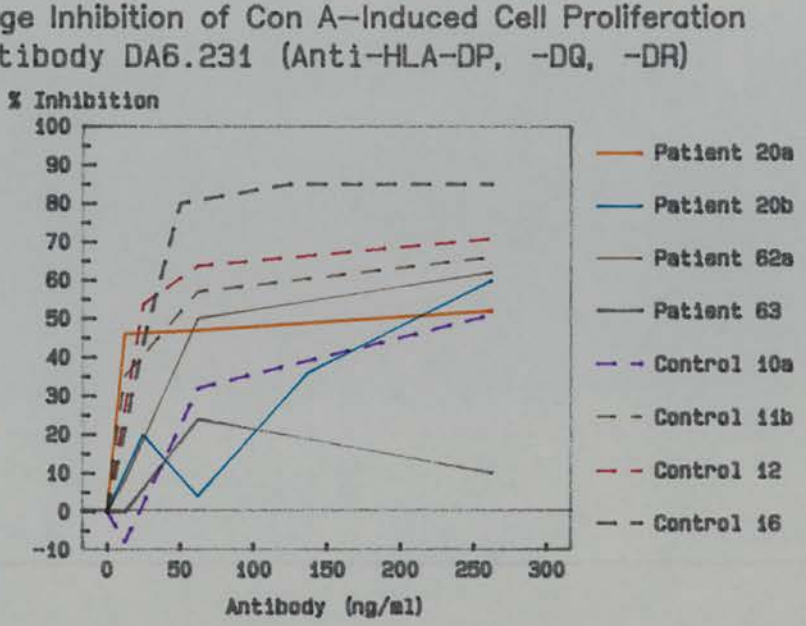
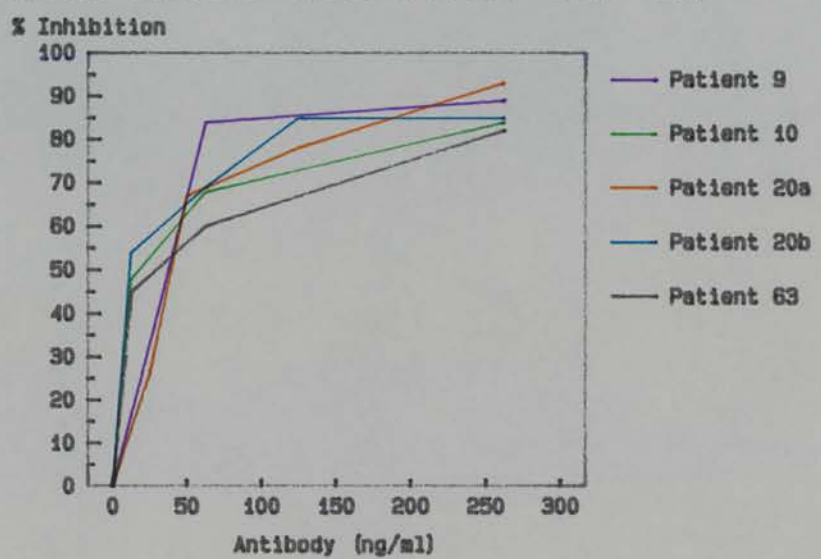


Fig 5.7b



PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml of DA6.231 (anti-HLA-DP, -DQ, -DR) and 10µg/ml Con A (Fig 5.7a, 4 patients, 4 controls) or 5µg/ml of $NiSO_4$ (Fig 5.7b, 5 patients) in the 6 day LTT. The percentage inhibition of proliferation was calculated (1- [(cpm with antibody) / (cpm without antibody)]) x 100.

Percentage Inhibition of Con A–Induced Cell Proliferation
Antibody L243 (Anti-HLA-DR)

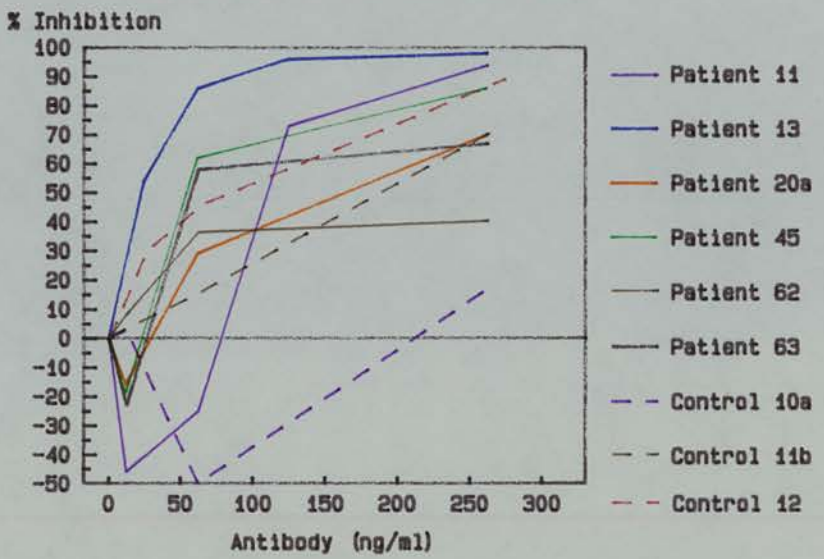


Fig 5.8a

Percentage Inhibition of Nickel Sulphate–Induced Cell Proliferation
Antibody L243 (Anti-HLA-DR)

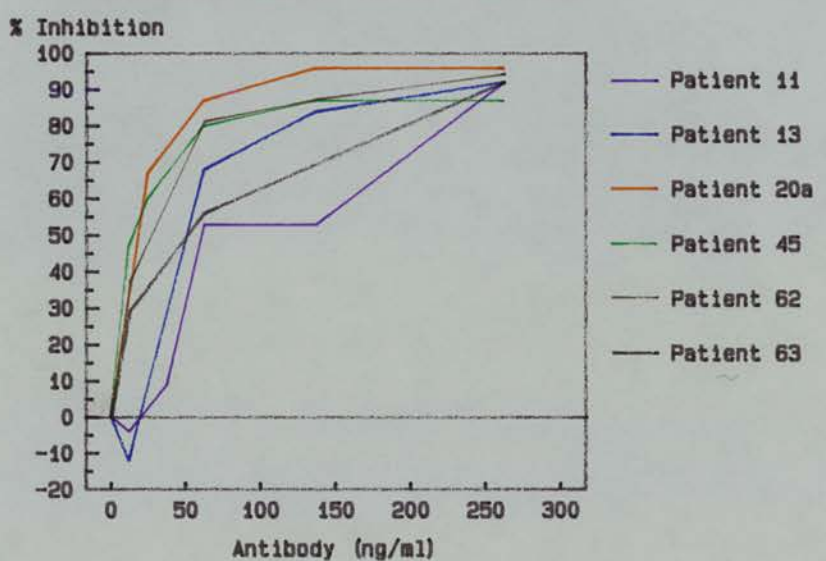


Fig 5.8b

PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml of L243 (anti-HLA-DR) and 10µg/ml Con A (fig 5.8a, 6 patients, 3 controls) or 5µg/ml $NiSO_4$ (fig 5.8b, 6 patients) in the 6 day LTT. The percentage inhibition of proliferation was calculated (1- [(cpm with L243) / (cpm without L243)]) x 100.

Fig 5.9a

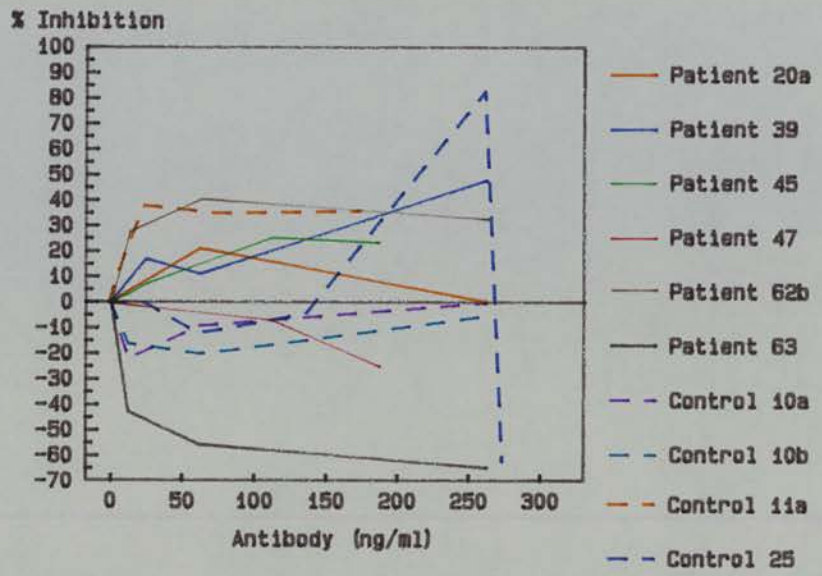
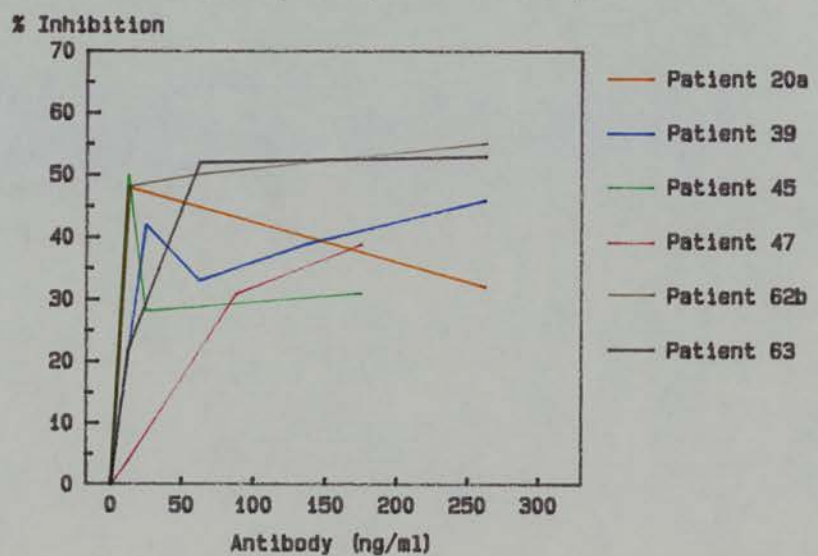


Fig 5.9b



PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml B7/21 (anti-HLA-DP) and 10µg/ml Con A (fig 5.9a, 6 patients, 4 controls) or 5µg/ml $NiSO_4$ (fig 5.9b, 6 patients) in the 6 day LTT. The percentage inhibition of proliferation was calculated (1 - [(cpm with B7/21) / (cpm without B7/21)]) x 100.

Percentage Inhibition of Con A–Induced Cell Proliferation
Antibody Leu 10 (Anti-HLA-DQ)

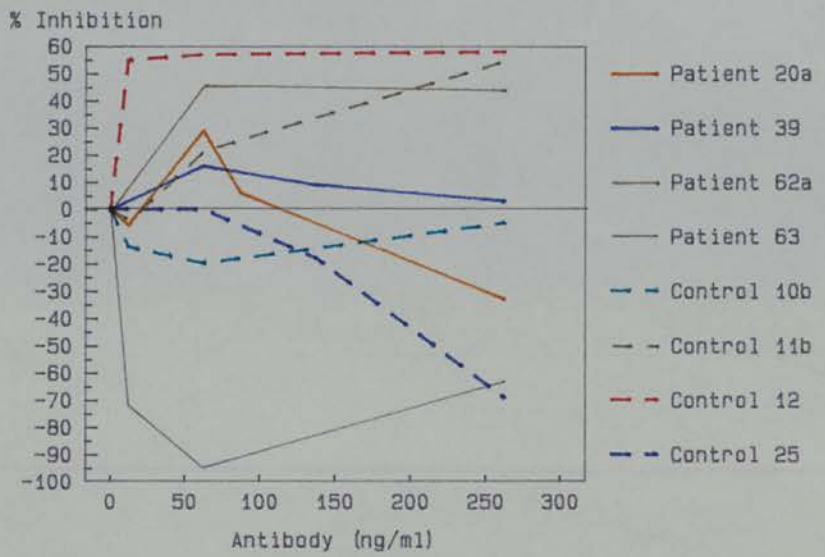


Fig 5.10a

Percentage Inhibition of Nickel Sulphate–Induced Cell Proliferation
Antibody Leu 10 (Anti-HLA-DQ)

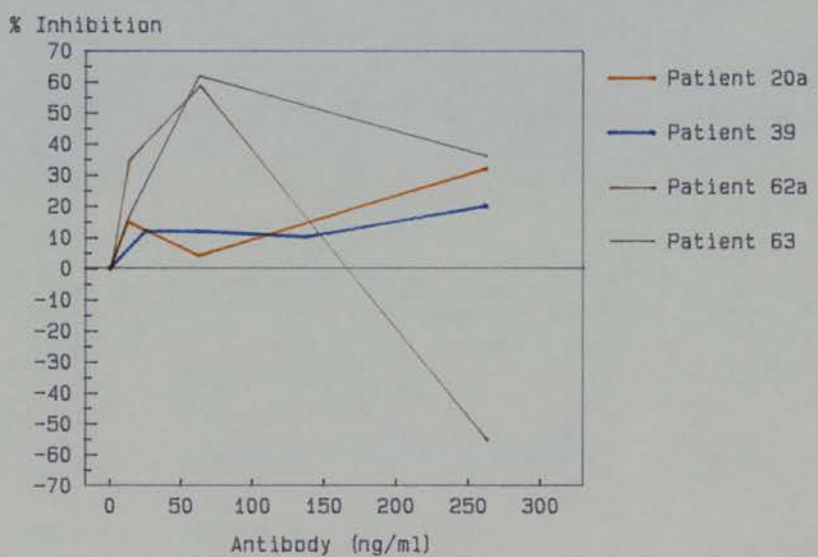


Fig 5.10b

PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml Leu 10 (anti-HLA-DQ) and 10µg/ml Con A (fig 5.10a, 4 patients, 4 controls) or 5µg/ml $NiSO_4$ (fig 5.10b, 4 patients) in the 6 day LTT. The percentage inhibition of proliferation was calculated (1- [(cpm with Leu 10) / (cpm without Leu 10)]) x 100.

Percentage Inhibition of Con A-Induced Cell Proliferation
Antibody L368 (Anti-β_2-Microglobulin)

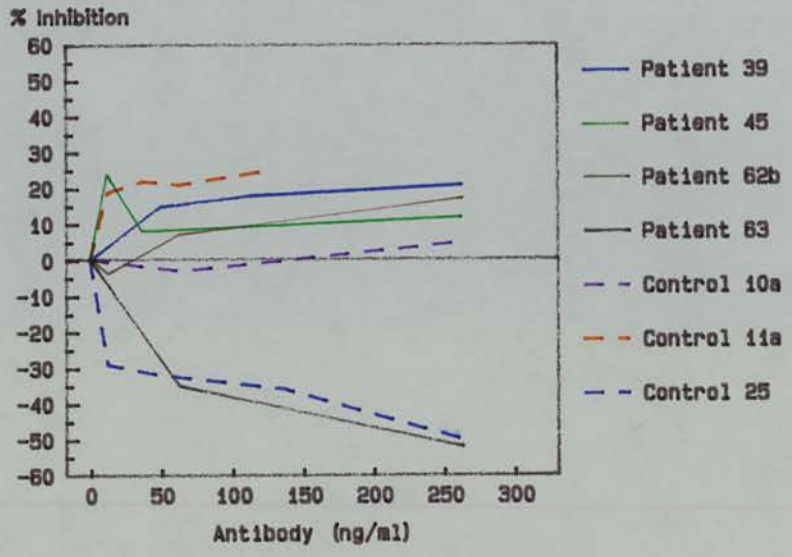


Fig 5.11a

Percentage Inhibition of Nickel Sulphate Induced Cell Proliferation
Antibody L368 (Anti-β_2-Microglobulin)

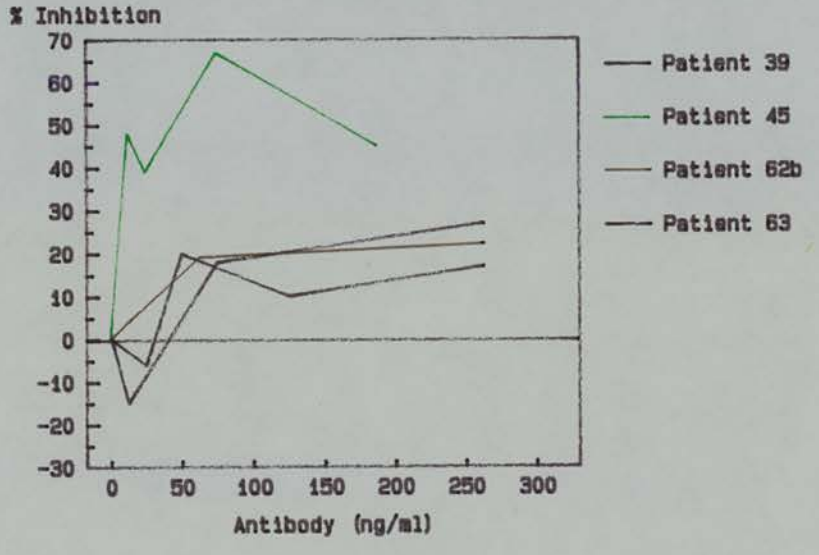


Fig 5.11b

PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml L368 (anti-beta$_2$microglobulin) and 10µg/ml Con A (fig 5.11a, 4 patients, 3 controls) or 5µg/ml $NiSO_4$ (fig 5.11b, 4 patients) in the 6 day LTT. The percentage inhibition of proliferation was calculated (1- [(cpm with L368) / (cpm without L368)]) x 100.

The Role of MHC Molecules of Different Antigen Presenting Cells in Accessory Cell Function

It was initially hoped to study the role of epidermal cell MHC molecules in antigen- and mitogen-induced T-cell proliferation. However, because of the limited availability of epidermal cells from nickel-sensitive patients, these experiments could not be carried out.

A study was possible, however, using plastic-adherent blood cells as APCs. These accessory cells were irradiated and then incubated with either DA6.231 (260ng/ml) or L243 (260 or 52ng/ml) for one hour before the addition of nylon-wool-enriched T-cells and either 10µg/ml of Con A or 5µg/ml of nickel sulphate.

The results obtained for both antibodies are shown in Appendices XII (DA6.231 = 3 patients, 3 controls, page 207) and XIII (L243 = 4 patients, 3 controls, page 208). Results obtained using cells incubated with DA6.231 (P11 and C12a) and L243 (P20a and C12d) are shown in figs 5.12-5.15. The percentage inhibition of cell proliferation by the antibodies is shown in Table 5.3, and the stimulation indices obtained with and without antibody are in Table 5.4.

As expected, the addition of 260ng/ml of DA6.231 to PBM inhibited the antigen-specific response of cells from patients 11 and 51 by more than 80% (see Table 5.3). Inhibition by the antibody was much weaker (37%) when enriched T-cells from Patient 1 were reconstituted with plastic-adherent blood cells. Inhibition of the Con A response by 260ng/ml of DA6.231 ranged from 23-87%, and was less marked when enriched T-cells were reconstituted with plastic-adherent blood cells.

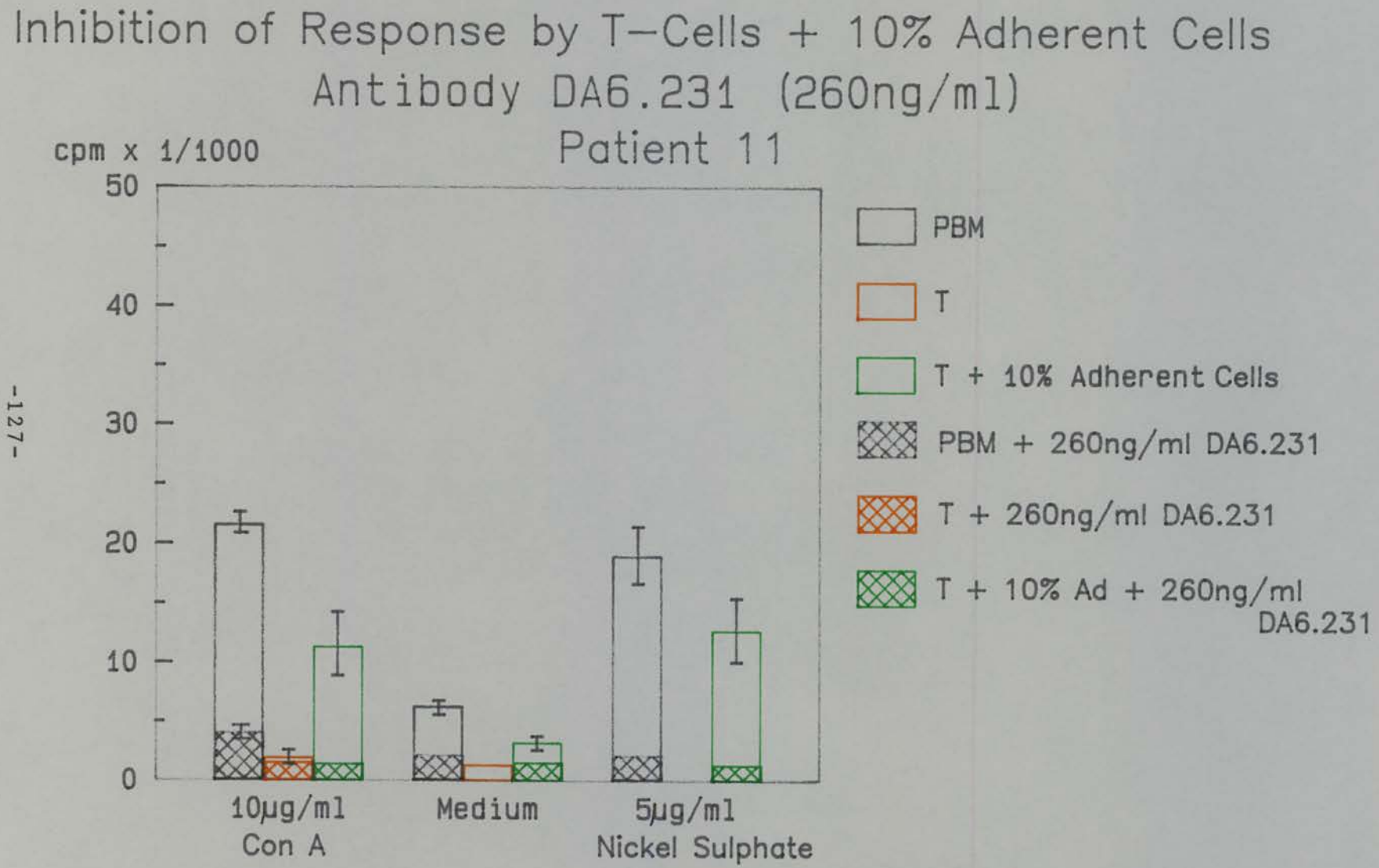
Table 5.3 Percentage Inhibition of Cell Proliferation using Antibodies DA6.231 and L243

	10µg/ml Con A				5µg/ml $NiSO_4$			
Ab	PBM		T +10% Ad		PBM		T + 10% Ad	
(ng/ml)	260	52	260	52	260	52	260	52
DA6.231								
Pat								
P1	77		47		83		37	
P11	81		87		88		89	
P51	81		73		92		95	
Cont								
C12a	76		40					
C12b	59		23					
C13a	58		48					
L243								
Pat								
P20a	74	47	65	51	84	34	90	56
P40	79	N.T.	83	N.T.	87	N.T.	96	N.T.
P48a	48	25	67	34	88	71	92	68
P50	83	64	76	56	58	42	81	75
Cont								
C12d	81	35	84	57				
C13b	60	N.T.	75	N.T.				
C27	59	24	70	50				

N.T. = Not Tested

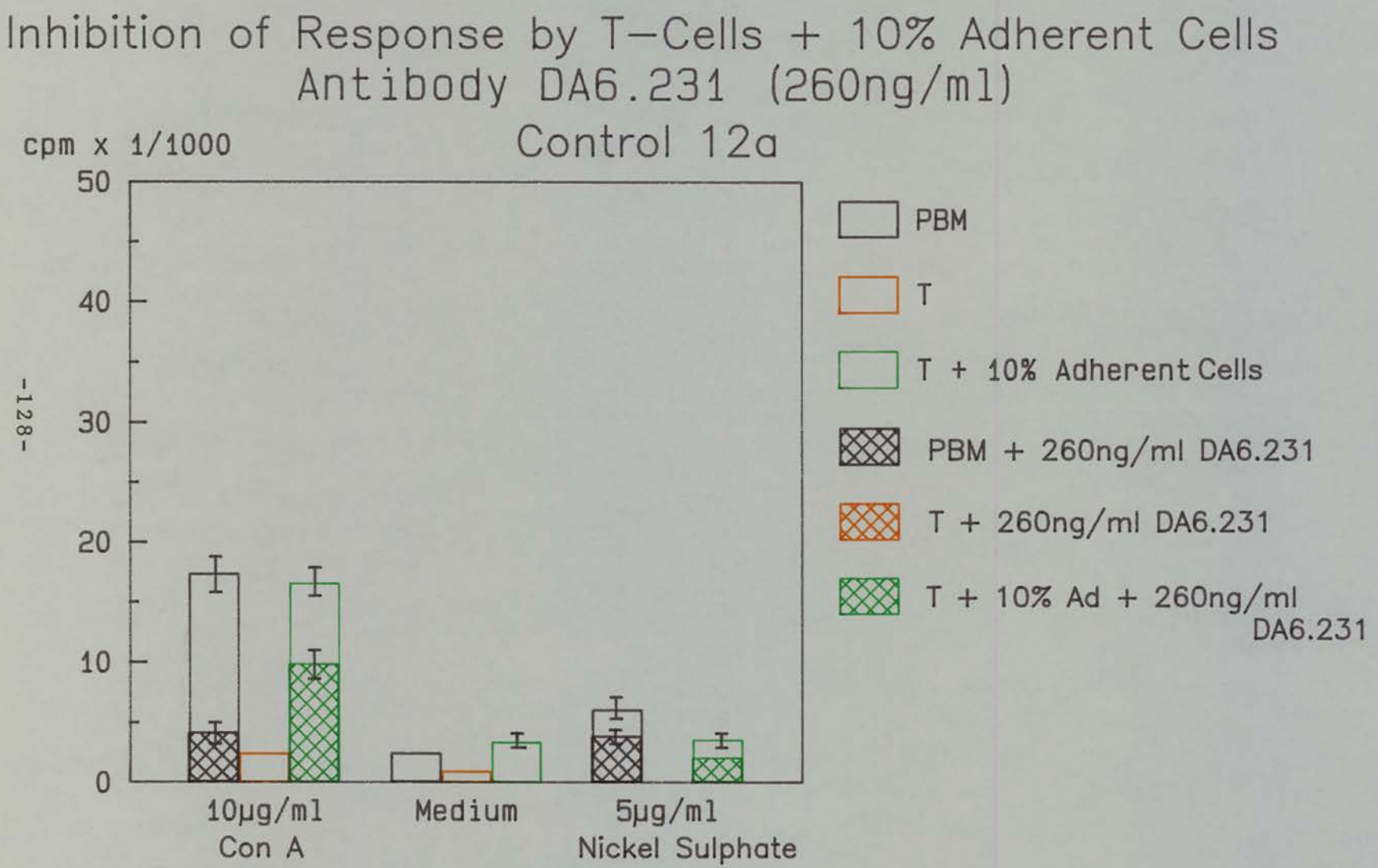
The addition of 260ng/ml of L243 also inhibited the proliferation in response to antigen and mitogen of both PBM and enriched T-cells reconstituted with plastic-adherent blood cells. In 3 of the 4 experiments carried out, the proliferation of the patients' cells in the presence of 5μg/ml of nickel sulphate was inhibited by more than 80% by 260ng/ml of L243 (Table 5.3). Addition of 52ng/ml of L243 inhibited the antigen-specific responses by more than 30%. The higher concentration (260ng/ml) of L243 induced greater inhibition of the Con A response than an equivalent concentration of DA6.231; more than 50% inhibition was evident for cells from the three nickel-sensitive patients and non-sensitised controls. The Con A response was less affected by 52ng/ml of L243, the inhibition being only 25-50%.

Fig 5.12



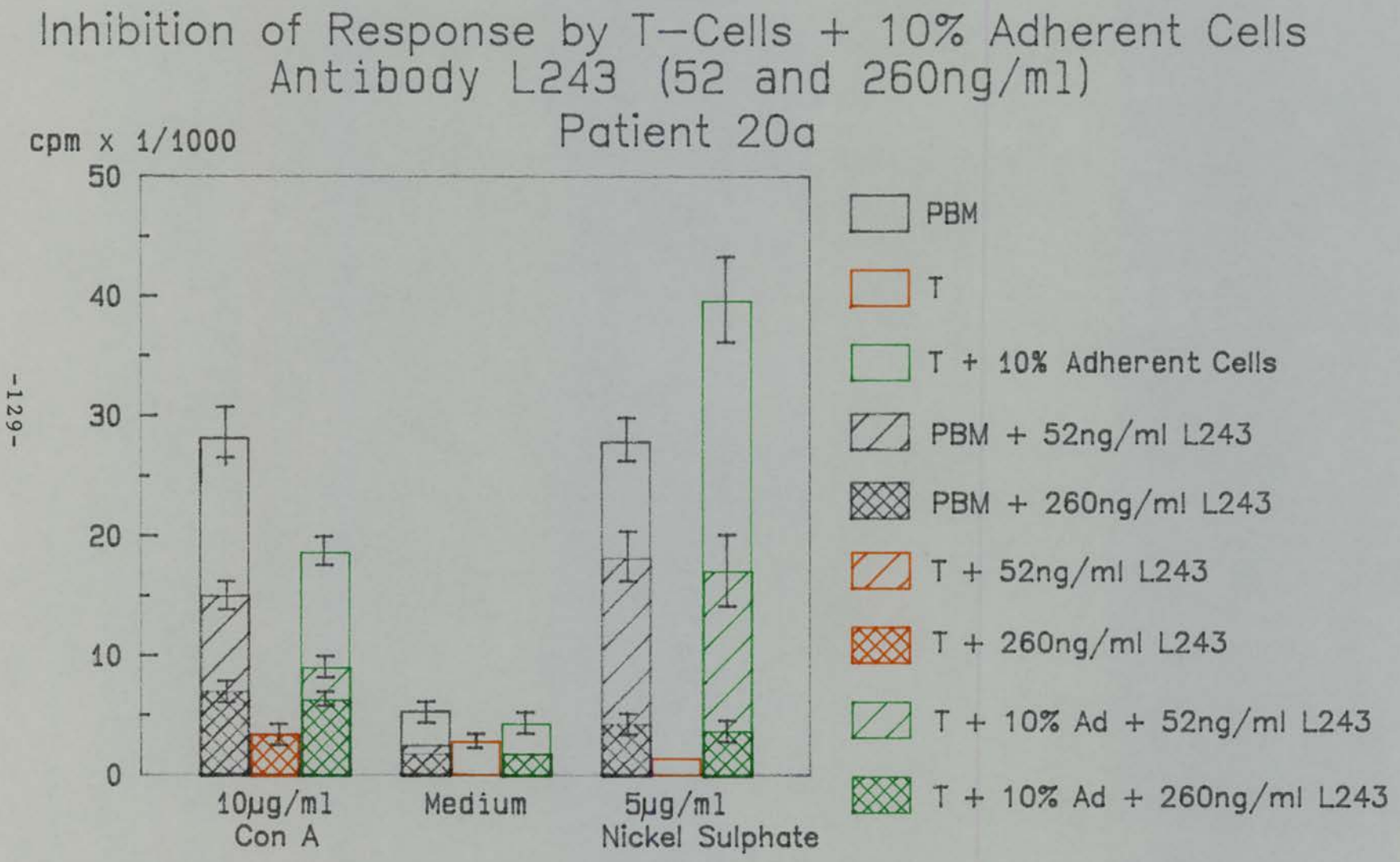
PBM and plastic-adherent blood cells from a nickel-sensitive patient were incubated with 260ng/ml of DA6.231 for 1 hour at room temperature before the addition of either Con A or nickel sulphate, and the addition of T-cells to the plastic-adherent blood cells. The 6 day LTT was then carried out. Results in cpm (Mean ± SD).

Fig 5.13



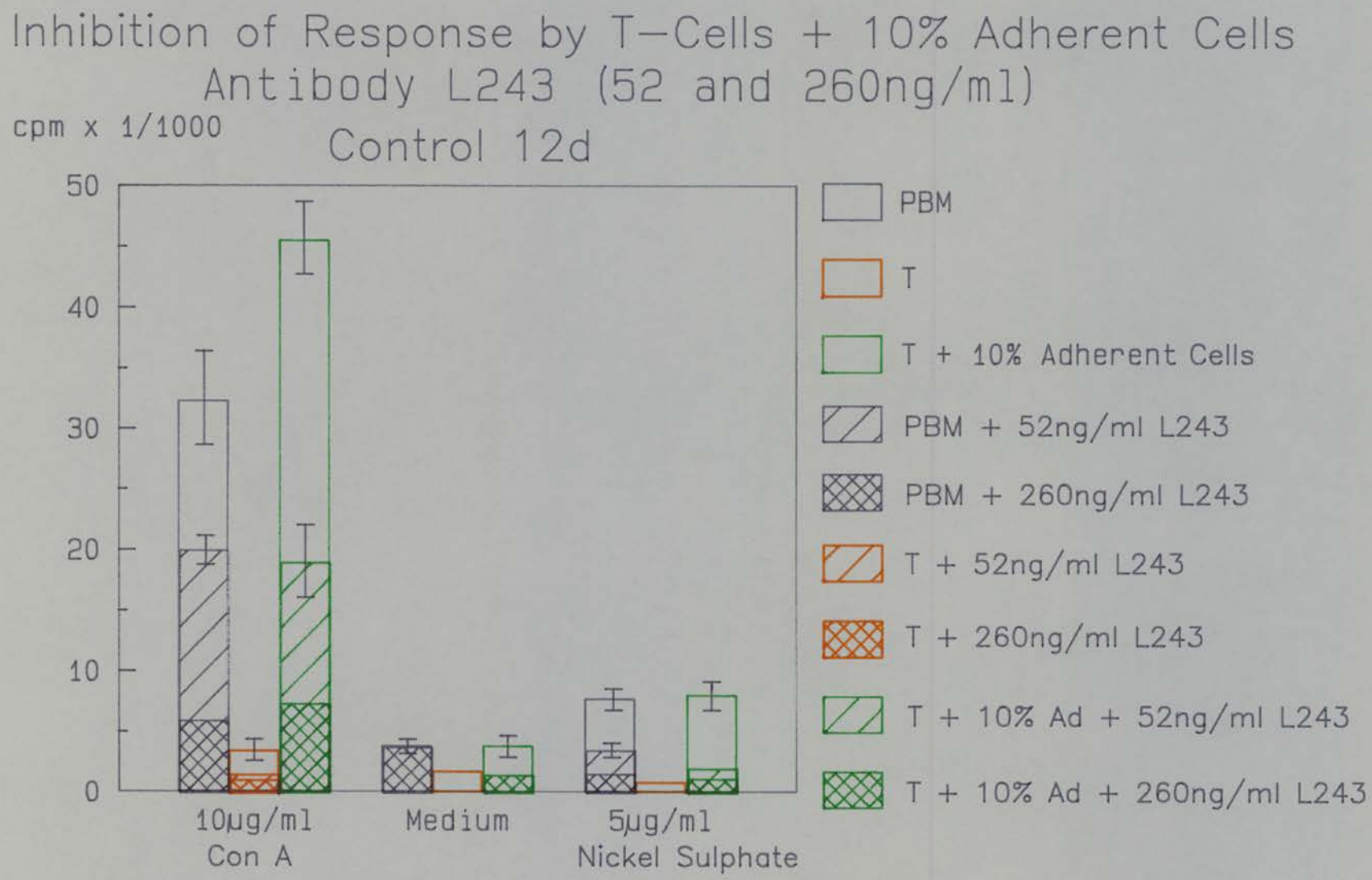
PBM and plastic-adherent blood cells from a non-sensitised control were incubated with 260ng/ml of DA6.231 for 1 hour at room temperature before the addition of either Con A or nickel sulphate and the addition of enriched T-cells to the adherent blood cells. The 6 day LTT was then carried out. Results in cpm (Mean ± SD).

Fig 5.14



PBM and plastic-adherent blood cells from a nickel-sensitive patient were incubated with 52 and 260ng/ml of L243 for 1 hour at room temperature before the addition of either Con A or nickel sulphate, and enriched T-cells to the plastic-adherent blood cells. The 6 day LTT was then carried out. Results in cpm (Mean ± SD).

Fig 5.15



PBM and plastic-adherent blood cells from a non-sensitised control were incubated with 52 and 260ng/ml of L243 for 1 hour at room temperature before the addition of either Con A or nickel sulphate, and enriched T-cells to the plastic-adherent blood cells. The 6 day LTT was then carried out. Results in cpm (Mean ± SD).

The background values of cell proliferation were also decreased by the addition of antibody to the cultures (see Appendices XII and XIII, pages 207-8). However, the stimulation indices obtained for cells from 12/13subjects (except Control 12b, see Table 5.4) incubated with Con A were reduced by the addition of 260ng/ml of either DA6.231 or L243. The stimulation indices obtained using cells from nickel-sensitive patients cultured with 5µg/ml of nickel sulphate were also reduced to values less than three in 6/7 experiments (see Patient 50, Table 5.4).

The results of these experiments indicated that the method used would be suitable for assessing the role in accessory cell function of MHC molecules on cells other than blood cells.

Table 5.4 Stimulation Indices Obtained from Cells Incubated with DA6.231 and L243 in Culture

	Con A						$NiSO_4$					
Ab	PBM			T + 10% Ad			PBM			T +10% Ad		
(ng/ml)	0	260	52	0	260	52	0	260	52	0	260	52
DA6.231												
Pat												
P1	6	4.9		3.6	2.8		4.1	2.5		3.2	3	
P11	3.4	1.9		3.5	0.9		3.0	1.0		3.9	0.9	
P51	20.8	4.0		17.5	6.9		12.5	1.2		15.0	1.2	
Cont												
C12a	7.4	2.9		5.4	4.2		1.5	1.7		1.1	0.8	
C12b	20.6	25.7		19.5	14.8		2.7	1.4		2.7	0.9	
C13a	3.8	1.8		4.9	3.6		2.3	1.2		2.0	0.5	
L243												
Pat												
P20a	5.2	3.6	5.6	4.3	3.3	4.8	5.2	2.3	6.8	9.0	2.0	9.1
P40	9.7	3.5	N.T.	6.2	1.3	N.T.	10.8	2.6	N.T.	15.8	0.8	N.T.
P48a	6.7	4.2	5.5	6.3	1.0	2.1	6.5	1.0	2.1	8.3	1.4	4.4
P50	22.9	10.3	5.6	13.6	4.5	15.3	4.1	4.7	1.6	6.0	1.6	3.9
Cont												
C12d	8.3	1.6	5.6	11.7	4.7	12.2	2.0	0.4	1.0	2.1	0.8	1.1
C13b	10.6	8.3	N.T.	6.4	1.8	N.T.	1.5	1.1	N.T.	1.1	1.1	N.T.
C27	5.4	4.3	6.5	2.4	4.1	10.3	2.6	0.5	0.8	1.4	0.7	2.0

N.T. = Not Tested

DISCUSSION

The aims of the study described were to establish an *in vitro* LTT which identified patients with ACD against nickel, and then to modify the assay to ascertain the accessory cell function of plastic-adherent blood cells and epidermal cells and the role of particular MHC II molecules in accessory cell function.

Detection of Nickel Sensitivity using the LTT

An *in vitro* LTT to detect nickel sensitivity was first published more than twenty five years ago (Aspegren and Rorsman, 1962) and a number of modifications recorded since then (see pages 38-41). Not all of the methods described discriminate successfully between nickel-sensitive individuals and non-sensitised controls. Antigen concentrations must be neither cytotoxic (Macleod *et al.*, 1970) nor mitogenic to lymphocytes (Svejgaard *et al.*, 1978; Al-Tawil *et al.*, 1981). In the preliminary studies reported in this thesis, investigations indicated that nickel sulphate concentrations greater than 30μg/ml were cytotoxic and that a concentration of 20μg/ml of nickel sulphate stimulated cells from certain non-sensitised individuals. It is unlikely that transformation of cells taken from "non-sensitised" controls indicates latent nickel sensitivity (Svejgaard *et al.*, 1978) because 25μg/ml of nickel sulphate has been shown to stimulate cord blood lymphocytes (Al-Tawil *et al.*, 1981), indicating a weak but significant mitogenic effect at higher nickel sulphate concentrations.

Proliferation of cells from nickel-sensitive patients and non-sensitised controls was therefore determined using 0-20μg/ml of nickel sulphate and with 10μg/ml of Con A in 6 and 7 day assays. As expected, cells from patients and controls were equally stimulated by the mitogen Con A (Svejgard *et al.*, 1978). Stimulation of cells from nickel-sensitive individuals by nickel sulphate was often dose dependent, being maximal with a

concentration of 20µg/ml of antigen in the 6 day assay and 10µg/ml of nickel sulphate in the 7 day assay. However, the greatest statistical difference between patient and control cell stimulation was obtained using 5µg/ml of antigen in the 6 day assay. The existence of two groups of nickel-sensitive patients whose cells respond maximally to either the highest or lowest nickel sulphate concentrations in the LTT (as described by Gimenez-Camarasa et al., 1975) was not evident amongst the patients studied using the assay described in this thesis.

Employing criteria of a stimulation index greater than or equal to three (Macleod et al., 1970) and a concentration of 5µg/ml of nickel sulphate in 6 and/or 7 day assays, cells from 61/66 (92%) patch test positive patients gave a positive response in the LTT; cells from none of the 43 negative controls responded.

Different antigen concentrations, incubation times and criteria for positivity have been employed by other investigators making direct comparisons of results difficult. However, the assay described in this thesis was more successful at detecting nickel-sensitive patients than those described by Macleod et al. (1970), Hutchinson et al. (1972), Svejgaard et al. (1978), Macleod et al. (1982) and Res et al. (1987), the major differences being the nickel sulphate concentrations employed and the use of only one 6 day assay. Assays carried out for different culture periods may increase the number of patients detected. Thus, in the experiments described in this thesis, a greater percentage of the patients studied using 5µg/ml of nickel sulphate were positive in the 7 day assay than in the 6 day assay.

False positive results were obtained by Svejgaard et al.(1978), Chu et al. (1987) and von Blomberg van der Flier et al. (1987). These may have been due to the mitogenic effects of higher concentrations of nickel sulphate used in the assays. False positive results described by Macleod et al. (1982) using a lower antigen concentration were unexpected as previous reports by these

authors (Macleod et al., 1970; Hutchinson et al., 1972) did not indicate that the concentration of nickel sulphate used in the assay (14μg/ml) would stimulate cells from non-sensitised controls.

Two assays have been described which were more successful in detecting nickel-sensitive individuals. Using a range of nickel sulphate concentrations, Gimenez-Camarasa et al. (1975) found that cells from 92% of 25 nickel-sensitive patients and none of 10 controls were positive in their 6 day assay. Al-Tawil et al. (1981) detected 15/16 (94%) patients and none of ten controls. They later reported 100% detection of 45 patients with no false positive results in 37 controls (Al-Tawil et al., 1985b, but their data was not shown). However, a recent report indicates that the assay may not be so reproducible (Gilboa et al., 1988). Furthermore, they used rather complicated criteria for positivity which required three nickel sulphate concentrations (including the mitogenic concentration of 25μg/ml of nickel sulphate) and a comparison of the results with those obtained using cells from controls.

Five nickel-sensitive patients were not detected by the assay described in this thesis. One patient (P51) had a +++ patch test reaction and may not have been detected because of the very high incorporation of tritiated thymidine into unstimulated lymphocytes taken from this individual in the 7 day assay. Three of the remaining four patients had weak (+) patch test reactions and may not have been truly allergic. Of 853 metal workers studied by Fischer and Rystedt (1985), 4 patients exhibited + reactions against nickel sulphate, but on retesting none of them gave a truly allergic reaction. Forman and Alexander (1972) have also described patients with positive patch tests and negative LTT results who when recalled were found to have no clinical signs of allergy and had become patch test negative. Therefore, some + reactions may not be truly allergic reactions and could be due to mild irritation.

A study of six patients indicated that PBM taken from patients when they first attended the clinic for application of patches did not have a significantly different response in the LTT to PBM taken at the time of the day 5 reading. This is in agreement with the results published by Veien *et al.* (1979) who found that only oral ingestion of nickel and not patch testing could increase antigen-specific T-cell proliferation. This was not described by Al-Tawil *et al.* (1985b) who reported increased nickel-specific lymphocyte transformation using cells from patients after patch testing. However, they only used cells from two patients and two controls. In general, however, patch testing may not increase *in vitro* nickel-specific cell proliferation because nickel is widespread in the environment and consequently the skin is continuously challenged with this metal.

Despite the ability of the LTT to detect patients with nickel sensitivity, correlations between patch test reaction severity and the stimulation indices obtained in the 6 and 7 day assays were weak. Low correlation coefficients were also obtained by Al-Tawil *et al.* (1985a) who compared results using the *in vitro* LTT with an *in vivo* serial dilution test (in which patients were patch tested with varying concentrations of nickel sulphate). Discrepancies between patch test reactions and LTT results may be attributed to the selection of patients (ie patients who had recently had dermatitis) (Al-Tawil *et al.*, 1985a) and the ability of the antigen to penetrate through the skin. In addition, the chemicals used in patch testing and the LTT are seldom obtained from the same source.

Discrepancies between *in vitro* and *in vivo* results may reflect the limitations of *in vitro* assays. In a true ACD challenge reaction, antigen is carried from the skin (probably by epidermal Langerhans cells (Botham *et al.*, 1987)) to the draining lymph nodes, where there is an expansion of antigen-specific T-cells. Inflammatory cells (including T-cells, monocytes and basophils) are then recruited into the site of antigen application. However, in human *in vitro* assays, the cells used are usually taken

from peripheral blood and not from the antigen-reactive site or draining lymph nodes. Local retention of antigen-specific lymphocytes at reactive sites and a decreased number of specific cells in the peripheral blood may result in false negative results (von Blomberg-van der Flier *et al.*, 1987). Measurement of the proliferation of cells obtained from an allergic-reaction site (using a method described by Kapsenberg *et al.*, 1987) may provide a better correlation with patch test results, but it is clearly unsuitable for routine clinical use.

Infiltrating T-cells in a nickel sulphate-allergic reaction are primarily $CD4^+$ (Scheynius *et al.*, 1983; Gawkrodger, 1987). In the nickel sulphate LTT, the majority of proliferating cells are $CD4^+$; however, their proliferation requires the presence of $CD8^+$ cells which also slowly proliferate in the culture (Silvennoinen-Kassinen *et al.*, 1986). It is not known whether the cells proliferating in the LTT are of the same phenotype as the T_{dh} cell. Expression of the T200 protein (CD45) (Morimoto *et al.* (1985) may subdivide $CD4^+$ cells into two functional populations, one providing help in specific antibody responses and the other making IL-2 and mediating DTH and T-cell proliferation (Janeway *et al.*, 1988). However, experiments to detect this protein were not performed. Furthermore, the cells present in the *in vitro* assay are taken from a blood sample and cannot replace the repertoire of cells capable of reacting *in vivo*. In addition, the leucocyte separation procedure depletes the cells of basophils. A T-cell dependent release of vasoactive amines from mast cells has been described (Askenase and Van Loveren, 1983) and the resultant inflammation will contribute to the *in vivo* allergic reaction but will not be detected by the LTT.

In summary, the LTT using 5µg/ml of nickel sulphate in 6 and 7 day assays with a stimulation index greater than or equal to three can discriminate reproducibly between nickel-sensitive patients and non-sensitised controls. However, reactivity of cells in the *in*

vitro LTT correlated weakly with the severity of in vivo patch test reactions.

Although in vitro assays cannot mimic exactly an in vivo situation, the LTT can be manipulated to study directly particular aspects of the response. This was done to assess the abilities of both plastic-adherent blood cells and epidermal cells to act as accessory cells to purified T-cells in the LTT.

The Accessory Cell Function of Plastic-Adherent Blood Cells and Epidermal Cells

T-cells were successfully enriched in a number of experiments using a combination of adherence to glass and plastic and nylon wool passage. Background proliferation of the cells was decreased and stimulation by either Con A or nickel sulphate was ablated. In a small number of experiments, Con A- or nickel sulphate-induced proliferation of enriched T-cells in the absence of additional accessory cells indicated the presence of contaminating accessory cells within the 'T-cell' population. The plastic-adherence step removed both monocytes and dendritic cells. Passage through nylon wool depleted cells of B-cells (Greaves and Brown, 1974), further macrophages and a large proportion of the remaining dendritic cells (Nussenzweig and Steinman, 1980). A final step of glass adherence permitted the adherence of monocytes which can readily reattach to surfaces. Thus, any contaminating cells in the population of enriched T-cells were probably dendritic cells or B-cells. However, the presence of 5% B-cells in a T-cell population does not affect cell proliferation (Braathen, 1980). In the experiments described in this thesis, less than 1% of MHC II^+ cells in the enriched T-cell population could occasionally mediate a patient T-cell response to either Con A or nickel sulphate or both, indicating that the contaminating cells were highly efficient accessory cells (therefore probably dendritic cells, Van Voorhis et al., 1983). However, attempts to stain dendritic cells

specifically using the antibody RFD1 (Poulter et al., 1984) were unsuccessful.

On average, 55% (range 28-72%) of the plastic-adherent cells expressed MHC II molecules. Quantitative MHC II expression is related directly to antigen presentation (Beller, 1984; Kurt-Jones et al., 1985). However, two functional subsets may exist within the MHC II^+ monocytes, one mediating antigen-induced proliferation, the other with the capacity to induce allo-reactive mixed leucocyte reactions (Raff et al., 1980).

Blood monocytes can present antigens but it is considered unlikely that ingestion and processing as described for lysozyme or ovalbumin (Allen and Unanue, 1984; Kapsenberg et al., 1986), is required for small antigens such as nickel. However, Silvennoinen-Kassinen et al. (1987) have shown that during the first five hours of culture it is only adherent cells which take up nickel. Furthermore, immediate uptake (within the first five minutes) was significantly greater using cells from nickel-sensitive patients than from controls, although uptake by both patient and control cells equalised after fifteen minutes.

Addition of plastic-adherent blood cells to enriched T-cells reconstituted the responses of nickel-sensitive patients' cells to both Con A and nickel sulphate and of T-cells from non-sensitised controls to only Con A. In general, SI obtained using T-cells reconstituted with plastic-adherent blood cells appeared to be less than those obtained using PBM, but they were not statistically different. This may reflect the removal of a subpopulation of cells required for maximum stimulation or the use of an insufficient number of accessory cells. The latter is unlikely because, in the experiments described, 10^5 plastic-adherent cells were added to 10^6 T-cells (10%) and this percentage of adherent cells has previously been shown to be an optimal concentration in LTTs using the antigens streptolysin O and staphylococcal protein A (Scala and Oppenheim, 1983). Unfortunately, many authors

investigating the role of different accessory cells in T-cell activation have not published stimulation indices obtained using PBM.

The plastic-adherent blood cells used as accessory cells contained monocytes and dendritic cells in unknown proportions. Reports indicate that approximately 5% of monocytes are dendritic cells (Van Voorhis et al., 1983). Differences in the staining of these accessory cells with antibodies DA6.231 (against MHC II antigens) and Leu M3 (against a monocyte marker) were minimal (see page 57); this may have resulted from variability in the staining and counting technique or have truly reflected the presence of 3% dendritic cells in the plastic-adherent cell population.

The precise roles of monocytes and dendritic cells in antigen presenting function have not yet been determined because of an inability to obtain a population with 100% purity. The relationship between dendritic cells and monocytes may depend on the antigen used in the system. Dendritic cells are required for the initiation of primary immune responses (Van Voorhis et al., 1983) and constitutively express high levels of MHC II molecules. However, they are weakly phagocytic and do not degrade particulate antigen (reviewed by Austyn, 1987). Therefore, small numbers of monocytes are obligatory for the presentation of particulate antigen by dendritic cells (Kapsenberg et al., 1986). Conversely, the presence of monocytes may inhibit dendritic cell presentation of soluble purified protein derivative (PPD) and it is thought that this inhibition of cell proliferation results from the release of prostaglandin E2 by monocytes (Bjercke and Gaudernack, 1985).

The ability of blood dendritic cells to present nickel sulphate to enriched T-cells has been described recently by Res et al. (1987). However their results differ from those previously reported in that they were unable to detect nickel-specific lymphocyte transformation using PBM from some nickel-sensitive patients. These differences may have resulted from the use of an

assay system which had not been correctly established. Furthermore, the nickel sulphate concentrations they employed have been found to be either mitogenic or toxic, both during the experiments described in this thesis and by other authors (Svejgaard et al., 1978; Al-Tawil et al., 1981). Finally, they indicated that a population containing monocytes and dendritic cells could present antigen in very low numbers (less than 5%) before an autologous mixed leucocyte reaction occurred; this differs from the results presented in this thesis and from those described by Braathen (1980), Braathen and Thorsby (1980) and Rasanen et al. (1986).

Thus, reports in the literature indicate that plastic-adherent blood cells do possess accessory cell function in the stimulation of lymphocytes by both Con A and nickel sulphate. However, in ACD reactions, it is unlikely that peripheral blood APCs are involved in initiating the reaction. Evidence suggests that after epicutaneous application of antigen, epidermal LCs take up the antigen, leave the skin via the lymphatics and then settle in the draining lymph node where they maintain a clonal proliferation of antigen-specific T-cells (Silberberg-Sinakin et al., 1980; Knight et al., 1985).

The ability of epidermal cells to mediate Con A- and nickel sulphate-induced proliferation of T-cells was investigated using cells from nickel-sensitive patients and controls. Using immunofluorescence, 2% of the epidermal cells were shown to express MHC II antigens in contrast to the 55% of MHC II^+ cells in the population of plastic-adherent blood cells. However, 40% (or even 20%) epidermal cells were as effective as 10% plastic-adherent blood cells in permitting T-cell proliferation in the presence of 10µg/ml of Con A. Indeed, addition of 40% epidermal cells to the enriched T-cells often enhanced the Con A response. Similar results have been obtained using guinea-pig T-cells reconstituted with 20% epidermal cells and stimulated with 5µg/ml of Con A in a 4 day assay (Scheynius et al., 1983).

Epidermal cells (40% and 20%) also enhanced the responses of nickel-sensitive patients' T-cells incubated with both 2.5 and 5μg/ml of nickel sulphate. False positive results (Chu et al., 1987) were not obtained in the assay. These findings support work published by Braathen and Thorsby (1983) who used a much higher ratio of epidermal cells:T cells (1:1 or 2:1). They showed that epidermal accessory cells were more potent than monocytes on a per cell basis. Lower epidermal cell:T cell ratios (ie 20% and 40% as used in the experiments described in this thesis) produced much lower stimulation (Braathen, 1980), although it is not known whether these epidermal cell concentrations fully reconstituted the T-cell response because results obtained using PBM or T-cells and monocytes were not published. Irradiated epidermal cells have also been shown to be more efficient than irradiated PBM at presenting influenza A and herpes simplex viruses (HSV) (Bagot et al., 1985).

For many years, the LC was considered to be the only epidermal cell capable of accessory cell function. LCs express MHC molecules (Rowden et al., 1977) and produce IL-1 (ETAF) (Sauder et al., 1984). LCs have been enriched using a number of techniques, including Percoll separation (Scheynius et al., 1983) and rosetting using the LC Fc receptor (Rasanen et al., 1986) or OKT6 (Bjercke et al., 1984). These LC-enriched fractions have been shown to possess accessory cell function.

A synergistic relationship between LCs and keratinocytes in accessory cell function was considered when keratinocytes were shown to produce IL-1 (Sauder et al., 1982). Subsequent studies have shown that keratinocytes express MHC II antigens in inflammatory conditions or in the presence of gamma interferon (Basham et al., 1984). These MHC II^+ keratinocytes may augment antigen presentation by LCs (Tjernlund and Scheynius, 1987; Scheynius et al., 1988), although they are unlikely to have played a role in the in vitro assay employed in this thesis because suction blisters were obtained from clinically uninvolved sites. Induction of keratinocyte MHC II molecules probably results from

the release of inflammatory mediators (including gamma interferon) by activated cells. It is possible that MHC II expression by keratinocytes may either maintain or down-regulate an ACD reaction.

At present, the relationship between LCs and blood dendritic cells is unknown. LCs are derived from the bone marrow (Katz et al., 1979) and therefore may be expected to migrate to the skin after transport in the peripheral blood. Thus, blood dendritic cells may be the precursors of LCs.

The capacities of enriched LCs and blood dendritic cells as APCs have been compared (Bjercke et al., 1985a; Res et al., 1987). Although both cell types present antigens, LCs may be more effective (Bjercke et al., 1985a). The reason for this increased accessory cell function is unknown. If blood dendritic cells are the precursors of LCs they may be less differentiated and therefore less efficient than LCs at acting as accessory cells (Kapsenberg et al., 1987). In addition, blood dendritic cells may be precursors of a number of cell types with different functions; in mice a Thy-1^+ dendritic cell has been described which is also derived from the bone marrow (Tschachler et al., 1983; Bergstresser et al., 1984) but which may down-regulate the immune response (Bigby et al., 1987). Furthermore, a small population of non-adherent $OKT6^+$ dendritic cells has been described in the peripheral blood (Dezutter-Dambuyant et al., 1984) and these cells may not have been isolated with the other blood dendritic cells but may be the true precursors of LCs and possess a similar accessory cell function.

The antigens commonly studied in vitro (eg nickel sulphate, PPD and HSV) are those which are presented to the immune system after passage through the skin. It is possible that after entry, the epidermal APCs may form a unique antigen recognisable by the T-cell but which is not formed by other types of APC. Thus, some nickel-specific clones derived from nickel-contact dermatitis lesions and expanded using mitogens, allogeneic cells and IL-2

recognised nickel only when it was presented by epidermal cells and not by monocytes and dendritic cells (Kapsenberg et al., 1987).

Finally, differences in the accessory cell functions of dendritic cells and LCs may result from differences in their abilities to induce either IL-1 or express MHC II molecules. Monocytes produce more IL-1 than LCs but are less potent accessory cells (Rasanen et al., 1986). Therefore, the expression of MHC II molecules may play a major role in accessory cell function. Although dendritic cells have a large surface area, LCs may express 50-100x more HLA-DR molecules than both blood monocytes and dendritic cells (Bjercke et al., 1985b). This increased number of LC MHC II molecules together with an absence of prostaglandin secretion may be responsible for the increased accessory cell function of LCs.

In summary, both plastic-adherent blood cells (monocytes and dendritic cells) and epidermal cells (probably LCs) from nickel-sensitive patients and non-sensitised controls could act as accessory cells in the presence of Con A. These accessory cells also stimulated the proliferation of T-cells from patients in the presence of nickel sulphate. The results obtained indicated that on a per cell basis, epidermal MHC II^+ accessory cells are more efficient than plastic-adherent MHC II^+ blood cells. The LTT was then modified further to investigate the role of the MHC II molecules in accessory cell function.

Role of MHC II Molecules

The requirement for MHC II^+ cells in antigen-specific T-cell proliferation is well documented (Habu and Raff, 1977; Thorsby et al., 1982). At present, three classes of MHC II molecules have been described in man, HLA-DP, -DQ, -DR. The molecules comprise an alpha and a beta chain. The HLA-DR alpha chain is constant in all individuals but amino acid differences are present in various HLA-

DP and -DQ alpha chain alleles, and all three beta chains are polymorphic. The variation in the molecules may result from requirements of alpha/beta pairing; the HLA-DR alpha and beta chains are apart on the genome and are more likely to be separated during recombination. Therefore, the alpha chain may be constant because it must be capable of joining a number of beta chains (Trowsdale, 1987).

It has been suggested that variability in the MHC II chains may reflect their functional differences, with different class II loci governing particular subsets of T-cells (Trowsdale, 1987). It was the aim of some of the experiments described in this thesis to determine whether or not expression of any of the three human MHC II molecules was important to accessory cell function in nickel-induced allergic contact dermatitis.

The requirement of 'self' MHC II molecules in antigen presentation was discovered by Rosenthal and Shevach in 1973 when they showed that antigen presentation by guinea pig macrophages could take place only if the T-cells came from an animal of the same strain. Similar experiments using cells from humans are harder to carry out because of the highly polymorphic nature of the MHC II antigens which can cause an allogeneic mixed leucocyte reaction, and the necessity to tissue type patients. However, a few studies have been described which show the necessity of 'self' HLA-DR molecules in antigen presentation. These employ antigen-specific T-cell blasts because addition of allogeneic cells to antigen-specific T-cell blasts produces a low mixed leucocyte reaction (MLR) in comparison to that obtained using resting T-cells.

In 1980, Bergholtz *et al.* showed that at least one HLA-DR determinant must be shared (or serologically cross-react) between the T-cell and macrophages to permit antigen-specific T-cell proliferation. Later, similar studies indicated that HLA-DR was not the only restricting molecule for antigen-specific T-cell

proliferation. HLA-DQ was shown to restrict presentation of PPD and HSV (Berle and Thorsby, 1982), and HLA-DQ and/or HLA-DP restricted presentation of contact sensitisers including nickel sulphate (Al-Tawil et al., 1985c). A number of experiments have shown that any of the three MHC II molecules can act as restriction elements for T-cell proliferation (Qvigstad et al., 1984).

As well as using tissue typed cells, the role of the different MHC II molecules in accessory cell function can be assessed using antibodies against one or more of the three MHC II molecules, and this method was used in the experiments described in this thesis.

Attempts to remove cells bearing MHC II molecules with the monoclonal antibody (DA6.231) and complement were unsuccessful. This probably resulted from the inability of the antibody to fix complement (because of its IgG subclass). Pulsing cells (ie incubating the cells with antibody and then washing off excess antibody before culturing the cells) partially inhibited nickel-specific cell proliferation when high concentrations of DA6.231 (6.5µg/ml) were used. Previous reports had indicated that pulsing cells with 2-20µg/ml of anti-MHC II antibody could inhibit antigen- or alloantigen-induced cell proliferation (Romagnani et al., 1985; Kalil and Wollman, 1983). However, pulsing cells with antibodies against HLA-DR may not always inhibit accessory cell function. MHC II antigens may be shed from the cell surface preventing persistent inhibition. Furthermore, pre-treatment of accessory cells may not interfere with the resynthesis and expression of class II antigens (Akiyama et al., 1985).

The limited availability of monoclonal antibodies prevented their use in the high concentrations required for cell pulsing. Therefore, an assay system was used in which lower concentrations of anti-MHC II antibodies remained in culture with the cells for the 6 day incubation.

Antibodies were added to the cultures at a concentration of 0-260ng/ml. These concentrations were acceptable for an experiment maintaining the antibody in culture, 100 ng/ml of soluble antibody being able to prevent antigen-specific cell proliferation (Muchmore et al., 1982).

HLA-DR

Addition of antibodies DA6.231 (anti-HLA-DP, -DQ, -DR) and L243 (anti-HLA-DR) directly inhibited the nickel-specific response of cells from sensitised patients and this inhibition was dependent on antibody concentration. These results support previous work suggestive of an important role for HLA-DR molecules in antigen presentation. Thorsby et al. (1982) showed strong inhibition of the PPD-specific proliferative response using antibodies against HLA-DR haplotypes common to autologous and allogeneic monocytes used as accessory cells. Pulsing monocytes with anti-HLA-DR for varying periods of time also inhibits antigen-specific proliferation (Muchmore et al., 1982; Gerrard et al., 1983; Romagnani et al., 1985). Finally, abolition of $HLA\text{-}DR^{+}$ cells with rabbit anti-HLA-DR and complement removes antigen-specific proliferation (Braathen and Thorsby, 1980).

Antibodies against HLA-DR initially bind to monocytes, macrophages, dendritic cells and B-cells. However, after activation, T-cells express MHC II antigens. The addition of antibodies two days after culture (after antigen presentation had taken place) did not affect T-cell proliferation and indicated that the decreased stimulation obtained in the presence of antibody did not result from direct inhibition of T-cell proliferation. Furthermore, cell viability was maintained after 6 days incubation with DA6.231 and L243 indicating that the antibodies were not cytotoxic.

Complement was present in the autologous plasma. Antibody L243 is of an IgG subclass capable of fixing complement.

Therefore, it is possible that some of the HLA-DR$^+$ cells were destroyed by antibody L243 and complement fixation. Antibody L243 gave slightly better inhibition of proliferation than DA6.231 but this could have been due to other reasons such as antibody affinity and avidity and the additional binding of DA6.231 to HLA-DP and HLA-DQ molecules.

The role of epidermal HLA-DR molecules in in vitro presentation of nickel sulphate was not investigated. It is likely though that they play a significant role in the induction of nickel sensitivity. Langerhans cells have been shown to express high numbers of HLA-DR molecules (Bjercke et al., 1985) and previous studies have shown that the removal of HLA-DR$^+$ cells with antibody and complement ablates antigen-specific T-cell activation (Braathen and Thorsby, 1980). Furthermore, experiments using tissue typed cells have shown that (like monocytes) epidermal cells and allogeneic T-cells must share at least one HLA-DR determinant to permit antigen-specific T-cell activation (Berle et al., 1982).

The roles of the other MHC II molecules in antigen presentation were less clearly defined by the experiments described in this thesis.

HLA-DP

The antibody B7/21 against HLA-DP appeared to inhibit the nickel-specific response only weakly. This inhibition may not have been specific as inhibitions greater than 50% were not always obtained. However, they were greater than those obtained using the 'control' antibody against beta$_2$microglobulin.

Antigen (influenza)-specific proliferation by an HLA-DP restricted T-cell clone has been described (Eckels et al., 1983). It is possible that only a few of the nickel-specific cells in the LTT were HLA-DP restricted, resulting in a weak inhibition of cell proliferation by B7/21. In addition, the percentage of cells

expressing HLA-DP molecules may have been very low. Neppert (1986) has shown that the staining of plastic-adherent cells (monocytes) with this antibody is about a quarter of the density shown by B-cells and concluded that HLA-DP may not be important in the functioning of monocytes. However, dendritic cells, which are considered to be highly efficient accessory cells, may express high levels of HLA-DP molecules when they have been maintained in culture for 24 hours (Brooks and Moore, 1988). Therefore, expression of HLA-DP molecules may have occurred later during the culture, permitting antigen-specific activation of HLA-DP restricted T-cells with an expected peak thymidine uptake on days 8 and 9 of culture, making inhibition of this proliferation less noticeable on day 6 which was the culture period used during these experiments.

HLA-DP antigens were discovered using HLA-A, -B, -C, -D and -Dw identical cells in the primed lymphocyte transformation test (Shaw et al., 1980). Using primary and secondary lympho-proliferative responses, $HLA\text{-}DP^{+}$ activated T-cells appear capable of inducing suppressor cells in normal lymphocyte populations and it has been suggested that these HLA-DP antigens may play a critical role in the negative feedback of the cellular immune response (Pawelec et al., 1984).

Thus, the type of cell expressing particular class II antigens may be as, or more, important than the presence of the molecules themselves. The role of epidermal cell HLA-DP molecules in in vitro nickel presentation has not yet been investigated. The observation that keratinocytes express HLA-DR ($L243^{+}$) and HLA-DP ($B7/21^{+}$) in a 48 hour nickel sulphate patch test reaction but not in an irritant reaction (Gawkrodger, 1987) may indicate an important role for keratinocyte HLA-DP expression in the induction, maintenance or suppression of an ACD reaction. Clearly, further work is required both in vitro and in vivo before the exact roles of HLA-DP molecules in accessory cell function can be determined.

HLA-DQ

Using the assay method described, antibody Leu 10 (anti-HLA-DQ) appeared to have no overall effects on nickel presentation. The results obtained were variable but further experiments could not be carried out because of antibody availability.

As described for B7/21, it is possible that Leu 10 had no effect on antigen presentation because an insufficient number of APCs were expressing HLA-DQ. After culture for 24 hours, monocyte expression of class II molecules decreases. However, dendritic cells may start to express a high density of HLA-DQ molecules on their cell surfaces which are maintained for at least 8 days (Brooks and Moore, 1988). In 1983, Gonwa et al. described an HLA-DQ^+ adherent cell subset which is responsible for the presentation of Candida albicans and is more efficient than an HLA-DQ^- subset in the stimulation of an autologous mixed lymphocyte reaction (AMLR), and it has been suggested that this results from the presence of dendritic cells in the HLA-DQ^+ population (Brooks and Moore, 1988).

Conversely, there is evidence to suggest that HLA-DQ molecules are important in the induction of suppressor cells. In 1980, Fainbom et al. showed that T-cells activated by mitogens or allogeneic cells for 11 or 23 days produced suppression when they were added to mitogen activated cultures. More than 40% of the $CD8^+$ cells expressed MHC II antigens. Furthermore, the suppression could be abrogated either by the removal of the $CD8^+$ cells or the addition of anti-HLA-DQw1 antibody (Festenstein and Ollier, 1987). HLA-DQ molecules on APCs may also be important in the activation of suppressor cells. Streptococcal cell wall antigen (SCW) and Schitosoma japonicum antigen (Sj) initiate poor immune responses in certain individuals because of the control exerted by an immune suppression (Is) gene in linkage disequilibrium with HLA-DR2-Dw12-DQw1 (Hirayama et al., 1986). Experiments using antibodies against MHC II haplotypes including HLA-DR2 and HLA-DQw1 have shown that the HLA-DR2 molecule presents the two antigens to $CD4^+$ cells.

However, this response is suppressed by suppressor T-cells controlled by the HLA-DQw1 molecule (Hirayama et al., 1987). A full in vitro response takes place when either $CD8^+$ cells are removed or anti-DQw1 is added to the culture (Hirayama et al., 1986).

Particular HLA-DQ molecules may therefore play a very important role in regulating the immune response by activating suppressor cells. However, it is unlikely that they play such a significant role in the induction or absence of nickel sensitivity. As seen in certain autoimmune diseases, population and family studies of nickel-sensitive patients have indicated a possible relationship between the incidence of nickel sensitivity and HLA-B haplotypes (Walton et al., 1986), but a relationship with MHC II molecules has not been described.

Mitogen-Induced Cell Proliferation

The requirement of MHC II molecules in mitogen-induced T-cell proliferation is less well defined. Experiments have been described in which T-cells proliferated in the presence of mitogen and a class II^- cell line (Bekoff et al., 1985). In this thesis, antibodies against HLA-DR (DA6.231 and L243) inhibited Con A-induced cell proliferation, but this inhibition was weaker than that seen in nickel-specific proliferation. In preliminary experiments, increased concentrations of DA6.231 did inhibit the Con A response more successfully, but an antibody concentration was chosen which did not totally abolish the mitogenic response because this helped to indicate that the cells were capable of proliferation in the presence of the antibody. The antibody against $beta_2$microglobulin did not affect Con A-induced cell proliferation; antibodies against HLA-DP and HLA-DQ had variable effects on Con A-induced cell proliferation and were inconclusive.

These results differ from those reported by Gerrard et al. (1983) and Akiyama et al. (1985) who showed that the monoclonal

antibodies they used against MHC II molecules do not affect Con A-induced cell proliferation. Gerrard et al. (1983) used the same antibody (L243) as described in this thesis. However, they pulsed the cells with antibody rather than allowing the cell to incubate with antibody throughout the culture period. It is possible that some of the antibody was shed from their accessory cells and that MHC II molecules were re-expressed, permitting Con A-induced cell proliferation. Although Akiyama et al. (1985) were unsuccessful at inhibiting mitogen-induced cell proliferation using monoclonal antibodies, some inhibition was obtained using rabbit antisera against class II molecules. These antisera would have contained antibodies against a number of determinants on MHC II molecules. However, their monoclonal antibodies, being so specific, may not have recognised an epitope important in antigen presentation. These reports illustrate that significant differences can be obtained depending on the different antibodies used.

In the experiments described in this thesis, the antibody against beta$_2$microglobulin did not affect Con A-induced cell proliferation. This is at variance with the results of Akiyama et al. (1985) who described inhibition of mitogen-induced cell proliferation using antibodies against both MHC I antigens and beta$_2$microglobulin. They suggested that both class I and class II antigens play a crucial role in the events resulting in proliferation after interaction with lectins. However, the results in this thesis and the finding that mitogenic lectins specifically bind to MHC II molecules (Kimura and Ersson, 1981) make their explanation unlikely. It is, however, possible that the different antibodies used in these two studies reacted with different determinants on the beta$_2$microglobulin.

Mechanisms of Inhibition

In conclusion, the experiments described in this thesis indicate that antibodies against HLA-DR can inhibit antigen-specific (and in some circumstances, mitogen-induced) cell

proliferation. However, the mechanism of this inhibition is presently unknown. T-cell activation requires a number of signals and has a number of stages. Therefore, the antibodies against class II molecules could exert one or many effects to prevent cell proliferation.

A number of mechanisms of inhibition have been investigated. Palacios (1982) showed that IL-2 production was arrested by the addition of anti-class II molecules, which indicated that T-cell activation and proliferation is stopped before IL-2 production, RNA and DNA synthesis and mitosis. Inhibition of the production of IL-1 (the 'first messenger' in accessory cell function) by the antibodies is unlikely as addition of exogenous IL-1 does not restore antigen-specific T-cell activation (Gerrard et al., 1983). Finally, the addition of certain antisera against HLA-DR may induce $CD8^+$ cells which non-specifically suppress the antigen-specific proliferation of cloned T-cells (Lamb et al., 1982). However, this is unlikely because the antigen-specific response can be restored by adding monocytes to cultures previously inhibited by the addition of monocytes pulsed with anti-HLA-DR (Gerrard et al., 1983).

The finding that pulsing cells with anti-MHC II antibodies inhibits antigen presentation by cells already coated with antigen indicates that anti-MHC II antibodies do not inhibit antigen binding or processing by the APC (Pawelec et al., 1985). Binding of immunogenic antigen to MHC II molecules has been described (Babbitt et al., 1985) and it is thought that the antigen must associate with an MHC II molecule for recognition by the T-cell receptor. Furthermore, a nickel binding fraction from PBM has been described which is a protein with a molecular weight similar to that of the HLA-DR beta chain (Silvennoinen-Kassinen et al., 1987). If this nickel binding molecule is shown to be part of the HLA-DR molecule, the antigen recognised by the antigen-specific T-cell receptor may comprise nickel bound to the HLA-DR beta chain. Therefore, it is probable that the anti-MHC II antibodies inhibit

T-cell proliferation by sterically blocking the MHC II- antigen complex which is recognised by the T-cell receptor.

General Conclusions and Areas to Extend the Work

The 6 and 7 day LTTs using 5µg/ml of nickel sulphate and an SI greater than or equal to 3 is an excellent and relatively simple *in vitro* method for detecting nickel sensitivity; cells from 92% of nickel-sensitive patients were positive in at least one of the assays, compared with none of the cells from the non-sensitised controls. This *in vitro* assay may ultimately be suitable for replacing patch testing as a method for establishing nickel sensitivity.

Although the assay was capable of detecting nickel sensitivity, there was a weak correlation between *in vitro* stimulation and patch test reaction severity. With co-operation from patients and clinicians, it may be possible to correlate results obtained in the LTT with disease activity (and not just patch test reactivity) over a long time period to establish whether stimulation in the LTT reflects the patient's clinical presentation of ACD to nickel. Furthermore, a study of $CD4^+$ and $CD8^+$ cells could be carried out to establish whether suppressor cells directly play a role in the quiescence of the disease. Previous work by Silvennoinen-Kassinen *et al.*, 1986) suggested that it is the $CD4^+$ cells which are proliferating in the assay system. Examination of other markers expressed by the cells *in vitro* (including the T200 marker (Morimoto *et al.*, 1985)) may provide further information about the type of $CD4^+$ cells activated in the culture. These results could then be compared with the histological identification of the same markers in nickel-allergic patch test reactions.

Enriched T-cells were obtained from PBM using a three-stage procedure. Further analysis of the $CD4^+$ and $CD8^+$ cells in the

remaining T-cell population may indicate whether a particular T-cell subset had been removed.

Both plastic-adherent blood cells and epidermal cells from nickel-sensitive patients were capable of acting as accessory cells in the presence of Con A and nickel sulphate in the LTT. Cells from non-sensitised controls reacted only against Con A. The ability of enriched blood dendritic cells and LCs to present nickel sulphate to enriched T-cells have been described (Res et al., 1987). Establishment of a method which can efficiently isolate those cells with dendritic morphology from samples of blood and epidermal cells would enable the use of these enriched cells as accessory cells in the modified LTT described in this thesis.

The role of HLA-DP, -DQ and -DR displayed on PBM was investigated by studying the effects on accessory cell function of monoclonal antibodies against the three MHC II molecules. The results obtained suggested that HLA-DR was necessary for accessory cell function. The availability of the antibodies limited the experiments which could be performed and these studies should be extended. Furthermore, different monoclonal antibodies against any one of the MHC II molecules may recognise different determinants. Therefore, it is possible that the use of a larger battery of monoclonal antibodies may indicate which determinants on an MHC II molecule are necessary for recognition by the T-cell. However, different antibody affinities and avidities will confound the results obtained. Finally, nickel sulphate is presented to T-cells after passage through the skin. The experiments described have shown that epidermal cells from nickel-sensitive patients can activate autologous enriched T-cells in vitro. The role of the three MHC II molecules expressed by the epidermal cells should be analysed using a similar method to that described using plastic-adherent blood cells.

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APPENDIX I

Mixtures In The European Standard Battery of Allergens

Mercapto Mix 1%

Mercaptobenzothiazole 0.25%

N-Cyclohexyl-benzothiazylsulphenamide 0.25%

Di-beta-naphthyl-para-phenylenediamine 0.25%

Morpholinyl-mercaptobenzothiazole 0.25%

Naphthyl Mix 1%

Phenyl-beta-naphthylamine 0.5%

Di-beta-naphthyl-p-phenylenediamine 0.5%

Caine Mix 3%

Benzocaine 1%

Nupercaine 1%

Surfacaine 1%

Fragrance Mix 8%

Alpha amyl cinnamic alcohol 1%

Cinnamic aldehyde 1%

Cinnamic alcohol 1%

Oakmoss absolute 1%

Hydroxycitronellal 1%

Eugenol 1%

Isoeugenol 1%

Geraniol 1%

Thiuram Mix 1%

Tetraethyl-thiuram disulphide 0.25%

Tetramethyl-thiuram-monosulphide 0.25%

Tetramethyl-thiuram-disulphide 0.25%

Dipentamethylene-thiuram-disulphide 0.25%

Parabens Mix 15%

Ethylparaben 3%

Benzylparaben 3%

Butylparaben 3%

Methylparaben 3%

Propylparaben 3%

PPD Mix 0.6%

Phenyl-cyclo-hexyl-p-phenylenediamine 0.25%

Phenyl-iso-propyl-p-phenylenediamine 0.1%

Diphenyl-p-phenylenediamine 0.25%

APPENDIX II

Names and Addresses of Suppliers

Amersham International plc

PO Box 16, Amersham, Bucks HP7

Methyl-^{3}H thymidine

Bacteriology Department, University of Edinburgh

Teviot, Edinburgh EH3

Guinea pig complement

BDH Limited

Burnfield Ave, Thornliebank, Glasgow G46

Toluene

Analar nickel sulphate ($NiSO_4.7H_2O$)

Glycine

Zinc chloride

Magnesium chloride

Becton Dickinson

Laboratory Impex Ltd, 111-113 Waldegrave Rd, Teddington, Middlesex

Anti HLA-DQ (Leu-10)

Anti HLA-DP

Anti $Beta_2$-microglobulin

Leu M3

British Oxygen Company

150 Polmadie Rd, Glasgow G5

Industrial gas cylinders

Cappel Scientific Division

Cooper Biomedical Inc., 1 Technology Court, Malvern, PA 19355, USA

FITC-Rabbit anti-mouse IgG (H & L) $F(ab)_2$ fragment

Elkay Laboratories Ltd

Unit 2, Crockford Lane, Basingstoke, Hampshire RG24

Culture tubes and caps

Epitest Ltd Oy

PO Box 943, SF-00101, Helsinki 10, Finland

Finn chambers on Scanpor

Fenwal Laboratories

Division of Travenol Laboratories Inc., Deerfield, Illinois 60015,USA

Scrubbed nylon fiber

Fisons plc

Loughborough, LE11

Bis-MSB (1,4-Di.(2-methylstyryl)-benzene)

PPO (2,5-Diphenyloxazole)

Flow Laboratories Ltd

Woodcock Hill Industrial Estate. Harefield Rd, Rickmansworth, Herts WD3

Tissue culture plates

Flowpore D syringe filter holders (0.2my)

Lymphocyte separation medium

Fungizone

Foetal bovine serum

Gibco Ltd

PO Box 35, 3 Washington Rd, Paisley PA3

RPMI 1640

L-glutamine (200mM)

Penicillin-Streptomycin

Gentamicin

Trypsin solution (2.5%)

Sodium bicarbonate

Mckay and Lynn

2 West Bryson Rd, Edinburgh EH11

Sterilin sterile universal tubes, conical tubes and pipettes

BD syringes

Moredun Research Institute

408 Gilmerton Rd, Edinburgh EH17

Sheep Red Blood Cells in Alsever's Solution

Northumbria Biologicals Ltd

South Nelson Industrial Estate, Cramlington, Northumberland NE23

Skatron filter mats

Sigma Chemical Company Ltd

Fancy Rd, Poole Dorset BH17

Aminoethylisothiuronium (AET)

Concanavalin A

Heparin

2-mercaptoethanol

Para-nitrophenyl phosphate

Trypan blue

Anti-mouse IgG

Mouse IgG

Anti-mouse IgG-alkaline phosphatase conjugate

Trolab

Hermal-Chemie Kurt Hermann.D-2057 Reinbek b.Hamburg

Patch Testing Substances

Appendix III Dose Responses For Nickel-Sensitive Patients Using 6 And 7 Day LTTs

	DAY 6						DAY 7					
Pat	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
P1	65+2	0.9+0.5	26.5+5	20.6+3	16.6+4	11.0+1	20+6	4.2+2.3	68.0+18	47.8+15	29.1+13	18.6+4
P2	71+3	0.6+0.2	N.T.	N.T.	9.0+1	4.9+1	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P3	80+8	1.1+0.4	N.T.	N.T.	26.0+6	11.4+2	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P4	90+19	0.9+0.4	11.6+5	N.T.	26.0+6	15.1+5	9+5	4.2+1.6	N.T.	22.2+6	25.7+14	N.T.
P5	56+10	2.5+0.8	N.T.	N.T.	18.0+1	13.5+2	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P6	42+9	0.7+0.4	16.1+7	15.2+5	8.3+3	9.4+1	10+2	2.8+1.9	37.4+10	35.2+2	27.3+8	10.0+2
P7	92+6	4.1+0.6	18.5+3	22.2+4	26.2+2	14.0+3	8+1	4.4+2.1	59.1+9	102+12	89+14	70.6+15
P8	73+15	0.8+0.3	14.4+2	14.9+0	6.7+2	2.0+0	17+2	0.4+0.1	36.0+3	40.1+9	30.2+11	5.0+1
P9	117+15	1.0+0.1	19.6+3	13.8+1	12.8+1	6.6+0	12+1	3.0+1.4	50.1+6	41+10	16.4+7	17.9+2
P10	98+10	1.8+0.6	28.0+5	18.4+2	13.1+2	6.8+2	17+3	3.3+1.3	44.7+10	43.5+8	51.7+19	13.2+1
P11	28+4	0.5+0.1	42.5+1	36.1+6	40.2+3	22.7+1	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P12	28+6	0.7+0.2	4.7+1	3.6+1	2.9+1	1.8+0	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P13	42+7	1.5+0.1	40.0+1	41.0+5	29.0+3	22.5+3	13+2	2.4+1.2	67.1+6	43.3+10	71.2+7	22.8+6
P14	11+4	0.5+0.1	N.T.	12.6+1	11.7+2	13.9+2	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.

Appendix III (continued)

Pat	DAY 6						DAY 7					
	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
P15	40+3	2.1+0.5	22.4+2	16.3+2	14.6+2	8.5+1	25+3	2.9+1.2	34.9+11	30.0+2	30.7+3	15.7+3
P16	59+3	3.7+0.5	17.2+1	12.3+1	7.6+2	6.0+1	60+6	3.8+0.6	30.3+6	26.0+4	17.7+4	12.9+2
P17	59+4	3.6+1.8	19.2+2	17.8+2	13.0+1	10.5+3	74+4	3.4+0.6	29.7+4	34.0+3	26.9+7	26.3+1
P18	52+5	5.2+0.8	57.7+4	38.2+12	29.8+9	9.3+5	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P19	122+2	8.1+1.1	64.1+6	51.4+7	39.3+3	21.6+2	46+5	7.9+2.3	98.0+13	101+20	84.2+20	63.7+6
P20	43+2	2.7+0.5	32.7+3	27.9+1	19.9+1	14.3+3	24+4	4.2+2	52.5+6	66.2+7	65.0+5	33.4+3.2
P21	113+10	3.8+1.2	23.6+3	16.1+4	12.6+2	7.0+0	11+1	7.4+1	64.9+5	55.7+15	43.1+12	18.2+3
P22	51+2	6.5+0.8	31.8+3	22.9+2	18.6+2	12.5+2	49+10	12.1+1	45.9+7	49.3+15	67.7+10	41.8+8
P23	35+5	2.4+0.6	83.5+22	37.0+13	45.1+19	31.9+4	56+2	4.3+1.5	51.2+3	91.1+4	60.1+3	42.0+5
P24	40+5	1.6+0.3	8.0+1	5.8+1	3.2+1	1.8+0	21+2	2.8+0.1	11.1+1	8.9+1	9.0+1	12.5+2
P25	66+6	3.5+0.9	26.1+3	22.6+5	22.0+1	8.7+2	15+3	2.6+0.8	28.1+4	36.4+4	42.5+6	17.5+6
P26	46+3	4.7+1.4	31.5+3	32.4+6	29.8+8	11.5+1	36+5	9.2+1.9	48.6+7	32.2+4	23.0+6	11.9+4
P27	5+1	1.4+0.2	34.2+3	40.4+5	46.3+5	25.9+3	4+1	1.5+0.7	19.4+1	48.4+5	43.5+10	38.4+7
P28	18+3	2.3+0.6	34.6+7	38.2+2	45.6+8	41.2+6	16+1	8.2+1.7	67.6+8	74.8+11	125+21	69.8+11

Appendix III (continued)

	DAY 6						DAY 7					
Pat	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
P29	23+2	2.6+0.7	38.8+2	38.7+6	41.4+7	26.5+6	11+1	7.6+4.0	48.4+10	103+9	98.1+14	75.7+13
P30	15+3	2.0+0.6	8.8+1	7.5+1	5.7+1	5.1+2	7+1	3.0+1.2	15.0+3	10.4+3	13.1+3	14.4+2
P31	77+17	2.2+0.5	9.7+1	4.7+0	6.7+1	3.2+0	11+3	3.9+1.5	13.6+4	11.1+3	13.8+3	8.6+3
P32	71+21	1.6+0.6	14.6+2	4.4+1	4.0+1	2.7+1	20+6	3.3+0.5	20.3+7	16.5+5	14.1+3	5.6+1
P33	59+10	2.8+1.0	14.4+1	18.3+4	9.5+1	7.9+0	17+4	7.5+1.4	21.2+2	36.1+5	23.9+4	21.3+7
P34	9+1	4.2+0.5	23.2+3	23.1+5	22.6+3	15.5+1	14+3	11.4+3.2	32.6+3	32.6+3	36.4+2	27.0+12
P35	13+2	3.0+1.5	12.4+1	10.0+1	9.3+1	7.7+1	6+1	4.3+1.1	19.4+1	19.9+2	15.3+4	11.2+2
P36	17+3	5.8+0.4	22.0+2	24.6+3	20.8+2	14.6+2	14+3	11.4+3.2	32.6+3	32.6+3	34.0+2	27.0+12
P37	53+8	8.1+0.5	32.7+2	34.0+8	36.4+9	34.7+6	20+5	12.1+5.1	33.3+3	58.0+11	58.9+13	37.9+20
P38	36+9	5.4+2.2	29.3+5	19.0+7	16.7+3	17.2+4	8+1	9.1+1.0	27.9+2	38.8+2	44.1+5	37.4+6
P39	58+12	7.3+1.1	31.4+3	31.8+2	27.0+3	22.3+2	48+4	12.4+2	47.9+2	66.7+8	69.4+10	63.3+2
P40	39+5	8.0+0.2	N.T.	64.3+11	43.9+7	42.1+5	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P41	40+5	12.0+2.7	N.T.	48.9+12	38.8+5	25.1+3	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P42	48+10	3.6+1.4	19.9+2	14.9+3	12.0+2	14.4+4	50+17	5.4+3.1	26.4+2	37.4+11	27.9+13	26.1+3

Appendix III (continued)

	DAY 6						DAY 7					
Pat	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
P43	42+8	5.6+0.3	20.4+3	21.6+2	24.9+3	16.3+5	41+8	3.6+2.3	36.1+2	25.9+3	36.1+11	40.8+12
P44	17+3	2.6+0.3	12.9+1	11.5+1	12.4+1	9.9+1	6+0	5.6+1.0	22.2+2	27.4+3	24.4+2	19.6+4
P45	14+2	4.9+0.8	17.8+2	16.2+1	17.7+1	13.4+3	12+3	6.2+2.2	N.T.	28.3+1	30.1+3	23.7+4
P46	10+1	1.8+0.1	20.5+2	14.9+1	11.7+1	8.9+1	8+2	7.2+0.7	19.3+3	17.9+4	15.1+1	18.9+4
P47	10+1	5.5+0.6	22.3+2	24.3+2	24.8+2	20.9+5	4+0	5.8+1.5	27.4+2	35.1+4	37.4+5	27.6+3
P48	34+3	5.1+0.7	N.T.	34.8+8	33.7+3	25.9+2	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
P49	28+3	6.8+2.4	21.3+2	20.0+2	16.4+2	17.0+1	15+1	7.5+1.1	30.0+6	33.5+3	22.6+4	28.0+4
P50	98+7	4.3+1.6	15.4+2	16.3+2	17.5+3	6.8+1	18+3	4.3+1.3	13.9+2	16.2+3	14.7+2	14.2+1
P51	48+11	2.3+1.0	N.T.	N.T.	28.9+4	N.T.	3+0	3.5+0.8	N.T.	75.5+2	48.6+7	24.2+3
P52	36+10	6.8+1.6	N.T.	17.2+3	14.6+2	12.5+3	18+5	14.2+1.4	N.T.	25.6+3	26.8+6	20.2+4
P53	19+4	5.3+2.0	13.0+1	13.0+5	11.3+1	7.1+1	N.T.	4.1+0	N.T.	13.3+4	11.7+2	N.T.
P54	11+1	2.6+0.8	22.8+1	29.2+4	31.2+5	23.8+5	6+1	4.4+1.5	22.6+2	47.2+1	52.2+3	50.9+4
P55	17+4	9.2+4.6	27.6+4	46.0+4	48.3+3	39.2+9	13+1	19.0+2	31.4+6	64.6+4	62.2+7	58.3+5
P56	44+5	5.8+1.4	N.T.	14.1+4	13.5+2	7.4+2	30+1	6.1+1.0	N.T.	22.1+7	16.6+4	14.2+2

Appendix III (continued)

	DAY 6						DAY 7					
Pat	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
P57	20+2	4.3+1.2	12.4+2	11.0+3	11.6+2	7.1+1	8+1	3.8+1.0	13.5+1	20.8+3	20.4+2	16.8+1
P58	13+1	2.5+0.6	14.5+1	10.8+1	6.9+0	4.5+1	7+1	8.7+2.4	15.4+1	20.4+1	17.9+3	15.7+3
P59	23+6	1.9+0.5	10.9+1	10.2+1	8.9+1	6.5+0	14+2	4.7+2.0	15.0+2	16.5+3	15.6+4	10.6+1
P60	39+4	2.9+0.6	12.6+1	10.0+1	9.7+0	6.7+1	21+3	4.4+1.4	15.8+1	17.1+2	15.6+2	12.7+2
P61	26+3	4.0+0.6	11.9+2	24.9+2	29.2+3	27.8+3	9+1	4.8+1.5	6.7+1	18.0+6	20.9+2	17.9+5
P62	42+6	3.7+1.4	12.8+2	15.2+2	12.5+1	10.2+1	10+3	3.2+1.7	10.1+2	24.6+5	31.1+6	20.7+5
P63	20+1	4.3+1.1	38.0+3	33.4+2	21.3+3	16.6+1	6+0	6.9+0.4	36.1+4	28.4+5	23.7+5	19.2+4
P64	25+1	4.3+0.4	15.6+2	9.4+1	7.7+1	5.9+1	8+1	6.1+1.0	21.6+3	15.8+4	8.7+2	8.3+1
P65	14+3	3.2+1.7	18.5+1	17.0+2	14.5+2	13.7+3	N.T.	4.0+1.8	N.T.	37.6+8	31.3+2	22.3+3
P66	5+1	1.6+0.3	29.6+3	32.8+1	33.9+3	26.6+3	6+1	3.3+0.2	32.9+3	51.6+5	38.4+8	21.5+6

Results in cpm x 10^{-3} (mean + SD)

N.T. = Not Tested

Appendix IV Dose Responses for Controls Using 6 And 7 Day LTTs

	DAY 6						DAY 7					
Cont	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
C1	21+7	2.6+1.1	5.3+1	2.7+0	2.6+1	2.6+0	29+5	4.7+1.2	9.2+1	4.4+0	2.2+0	2.7+1
C2	11+2	3.8+1.0	3.6+1	2.8+1	1.6+0	1.0+0	2+0	2.0+0.6	2.5+1	2.7+0	1.6+0	1.1+0
C3	12+2	2.9+1.6	12.3+4	7.3+1	6.0+1	4.3+0	6+1	2.1+1.0	10.3+1	5.9+1	5.2+1	N.T.
C4	44+5	5.0+0.4	19.0+1	18.4+2	7.7+2	11.2+2	40+3	10.4+3.0	20.0+2	24.0+2	15.2+1	23.9+3
C5	78+8	5.7+1.3	10.4+2	7.9+0	3.9+0	5.9+1	53+5	13.8+0.9	15.2+3	12.9+2	7.7+1	10.5+3
C6	27+6	7.3+1.1	8.0+1	6.8+1	3.7+1	5.1+1	20+3	12.8+4.0	14.3+2	9.6+2	11.1+3	14.4+1
C7	91+10	4.3+1.5	16.0+4	7.2+1	4.2+1	3.3+0	21+3	3.3+1.5	35.6+8	14.0+4	8.2+3	4.8+2
C8	129+17	1.2+0.2	N.T.	1.2+0	1.2+0	1.0+0	23+3	2.6+1.3	6.2+1	6.2+1	1.7+1	0.8+0
C9	12+2	1.1+0.7	0.9+0	1.0+0	1.0+0	1.0+0	13+1	0.7+0.3	0.4+0	1.0+0	1.0+0	0.9+0
C10	136+8	1.5+0.1	2.7+1	1.9+0	1.9+0	1.5+0	15+1	4.3+1.0	2.2+0	3.0+1	1.6+0	1.1+0
C11	88+7	1.1+0.2	5.0+1	2.9+1	1.7+0	1.5+0	16+1	2.9+0.5	10.5+1	4.1+1	5.5+1	2.6+1
C12	15+3	2.5+0.8	N.T.	3.0+0	1.1+0	1.9+1	19+7	5.1+0.5	N.T.	19.0+2	9.2+3	5.8+1
C13	53+4	1.5+0.3	5.3+1	3.1+1	3.1+1	2.4+1	7+3	3.4+2.3	18.8+5	1.8+1	9.3+1	4.9+2
C14	35+2	2.0+0.3	4.7+1	2.2+0	2.2+0	2.4+1	21+5	2.6+0.3	8.6+2	4.1+0	3.1+0	2.7+0

Appendix IV (continued)

	DAY 6						DAY 7					
Cont	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
C15	25+8	1.3+0.2	3.8+1	5.2+1	3.2+1	2.8+1	53+17	2.5+1.2	4.3+1	2.5+1	2.0+1	1.6+0
C16	145+10	4.3+0.5	42.3+1	7.8+2	3.2+0	2.6+1	33+2	6.8+1.1	33.4+2	22.7+2	14.5+3	6.5+2
C17	52+4	2.6+0.3	16.2+1	7.2+1	5.8+1	4.5+0	19+2	5.3+1.2	18.5+5	25.0+2	10.5+3	9.1+0
C18	16+1	2.5+1.6	7.8+1	4.9+2	4.7+0	4.5+1	3+0	4.0+1.0	8.6+2	8.6+3	5.5+1	N.T.
C19	23+2	1.8+0.9	N.T.	0.8+0	0.6+0	0.5+0	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
C20	21+5	2.8+0.5	2.8+1	2.6+1	2.5+0	2.6+1	7+1	2.2+0.5	6.5+2	1.9+0	1.3+0	1.8+0
C21	12+2	3.4+0.0	14.5+2	8.0+1	6.7+0	4.1+0	8+1	6.6+1.0	34.7+4	24.7+8	16.7+3	8.2+2
C22	42+11	4.7+1.7	11.6+3	6.1+1	5.0+1	3.0+1	8+1	6.6+1.0	N.T.	15.1+1	9.4+2	9.7+2
C23	23+4	11.1+.3.0	16.4+2	16.3+5	13.2+2	9.1+2	15+2	10.4+3.0	23.9+2	29.9+5	15.1+5	15.1+1
C24	43+4	7.5+1.6	13.9+2	12.9+2	9.9+3	8.3+4	21+3	14.6+4.0	27.6+7	26.7+5	15.1+4	8.9+4
C25	49+7	4.2+1.1	6.7+1	6.7+1	5.3+1	4.1+1	4+0	11.8+1.0	19.8+2	15.0+3	12.9+2	11.0+0
C26	31+4	5.7+0.6	N.T.	11.8+5	5.0+1	6.8+1	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
C27	31+4	5.7+1.5	N.T.	15.0+2	14.7+1	12.1+2	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
C28	28+5	8.5+0.3	N.T.	8.6+1	7.1+1	3.9+1	15+6	3.1+0.6	N.T.	11.9+1	7.0+2	5.8+1

Appendix IV (continued)

				DAY 6						DAY 7		
Cont	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
C29	28+4	10.6+2.2	17.3+2	10.2+2	10.3+2	10.0+1	18+3	22.6+2.0	23.0+3	24.0+2	22.6+5	22.1+3
C30	47+7	8.9+3.5	N.T.	12.9+3	11.0+2	7.6+2	20+5	9.2+1.8	N.T.	19.1+2	16.2+4	12.3+4
C31	18+3	2.6+0.7	4.8+1	4.5+1	3.2+0	4.3+2	10+0	2.5+0.8	7.1+1	5.9+1	3.3+1	4.5+1
C32	47+3	2.4+0.5	8.1+1	7.1+2	4.1+0	3.2+1	41+5	4.9+1.5	15.2+1	10.7+2	8.9+3	7.5+2
C33	10+1	4.6+0.4	7.2+1	5.8+0	4.2+1	3.3+1	9+1	10.6+2.7	12.4+1	13.6+4	5.6+1	10.8+2
C34	25+5	3.6+1.5	12.9+4	4.0+2	4.8+1	5.0+1	14+4	6.1+2.4	20.2+2	15.6+3	9.3+2	6.7+1
C35	9+2	2.4+0.8	4.8+1	3.5+0	3.0+0	1.8+0	7+1	3.7+1.4	7.5+1	3.3+1	3.3+1	4.8+1
C36	23+1	4.5+1.0	9.2+1	6.7+2	4.4+1	4.0+1	11+1	7.5+1.4	16.8+2	14.9+2	10.6+2	9.9+1
C37	68+9	4.4+1.7	10.0+1	7.0+1	3.7+1	3.2+1	27+3	7.8+3.0	18.8+4	13.6+3	10.0+2	8.8+1
C38	29+4	2.2+0.4	8.8+1	6.1+1	4.4+1	4.0+1	14+3	5.7+1.5	16.3+2	9.6+3	7.5+1	6.4+1
C39	23+3	2.0+1.2	8.1+2	6.6+1	2.6+0	3.4+0	12+1	4.9+2.1	16.4+1	9.6+2	7.3+1	5.6+1
C40	14+2	3.8+1.0	8.3+1	7.5+1	5.0+1	3.0+1	14+3	6.9+1.7	17.9+1	10.6+2	11.9+3	7.0+1
C41	33+5	3.1+0.1	8.2+1	4.4+1	3.5+1	2.9+0	16+2	5.6+1.1	14.1+1	9.9+2	7.3+1	10.3+4
C42	28+11	5.3+2.1	7.5+1	6.0+1	3.8+1	2.7+0	15+3	4.0+0.5	10.6+1	9.9+1	7.1+2	6.4+1

Appendix IV (continued)

	DAY 6						DAY 7					
Cont	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	20µg/ml $NiSO_4$	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
C43	11+3	2.1+0.6	8.4+1	4.7+1	3.7+1	2.7+0	8+2	3.9+1.1	14.6+3	10.8+2	6.6+2	4.0+1
C44	8+3	3.5+1.4	4.6+1	2.8+0	1.1+0	1.8+0	5+1	2.9+0.6	8.5+2	3.6+1	4.5+1	3.7+1
C45	25+6	3.6+1.4	12.8+4	7.3+3	7.3+2	3.3+1	11+1	4.2+1.5	13.4+2	12.8+3	9.1+5	8.1+2
C46	70+9	3.6+1.5	13.4+2	9.5+1	4.4+1	4.4+1	49+9	6.2+0.8	21.3+5	16.9+2	15.0+1	9.2+1

Results in cpm x 10^{-3} (mean + SD)

N.T. = Not Tested

Appendix V Proliferation of PBM and Enriched T-Cells With or Without Added Plastic-Adherent Blood Cells in the Presence of Con A or Nickel Sulphate

	PBM					T				T+10 %Ad				
Pat	10µg/ml Con A	Medium	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	5µg/ml $NiSO_4$	10µg/ml Con A	Medium	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
P1	37+6	6.1+0.9	N.T.	25.1+2	N.T.	2.4+0	1.6+0.5	N.T.	13+1	3.5+0.8	N.T.	11.4+1	N.T.
P3	80+8	1.1+0.4	N.T.	26.0+6	11.4+2	1.0+0	0.4+1.3	0.3+0	21+3	0.2+0.1	N.T.	11.2+3	4.3+1
P4a	90+19	0.9+0.3	N.T.	26.0+5	15.1+5	2.0+0	0.8+0.5	0.7+0	9+1	0.6+0.3	N.T.	0.9+0	0.7+0
P4b	56+9	2.5+0.7	N.T	18.0+1	13.5+2	2.1+0	0.6+0.1	0.6+0	7+2	0.3+0.1	N.T.	4.3+2	3.3+1
P9	34+10	3.0+1.4	19.2+2	12.3+3	N.T.	1.6+0	1.0+0.4	0.8+0	2+0	0.8+0.2	N.T.	1.4+0	N.T.
P12	22+2	6.3+0.9	18.5+2	19.0+4	N.T.	2.0+0	1.4+0.3	2.1+0	11+4	3.3+0.9	14.3+2	12.8+3	N.T.
P14	11+1	0.5+0.1	12.6+1	11.7+2	13.9+2	1.3+0	0.6+0.0	0.4+0	3+0	0.8+0.1	N.T.	4.6+0	N.T.
P18	14+2	5.0+1.9	N.T.	22.9+1	16.7+3	1.7+0	1.3+0.2	1.3+0	7+1	2.3+1.0	N.T.	19.1+2	12.3+1
P20a	28+3	5.4+1.5	25.6+2	27.9+2	33.7+2	3.5+1	2.9+0.9	1.5+0	19+2	4.4+1.1	37.6+4	39.7+4	42.3+4
P20b	28+2	6.3+1.5	56.6+7	56.6+6	52.5+5	11+1	7.3+0.8	27+11	20+4	5.5+1.1	72.7+7	67.9+3	72.3+5
P40	39+5	4.1+0.7	64.3+10	43.9+7	42.1+5	2.8+0	1.2+0.6	N.T.	29+8	4.6+1.1	76.8+15	73.1+6	75.2+8
P41	40+5	12.2+2.7	48.9+11	38.8+5	25.1+3	3.4+0	2.5+0.5	N.T.	21+4	9.6+1.5	38.9+3	34.4+3	N.T.
P46	13+1	3.5+1.4	13.5+3	13.4+1	10.6+2	4.5+1	2.8+1.0	2.5+1	28+4	3.3+0.7	17.6+4	12.3+1	10.7+2
P48a	34+3	5.1+0.7	34.8+3	33.7+3	25.9+2	7.8+4	4.3+1.6	20.9+5	26+3	4.1+1.0	31.7+5	34.1+1	34.3+3
P48b	26+3	4.2+1.5	19.7+3	26.2+3	19.8+1	3.2+1	2.4+0.8	4.2+0	26+3	4.5+0.7	33.1+4	32.2+3	27.3+5
P50	98+6	4.3+1.6	16.3+2	17.5+3	6.8+1	7.3+2	3.6+1.1	6.4+1	60+6	4.4+0.7	23.5+6	26.6+5	19.6+2

Appendix V (continued) Proliferation of PBM and Enriched T-Cells With or Without Added Plastic-Adherent Blood Cells in the Presence of Con A or Nickel Sulphate

	PBM					T				T+10% Ad			
Pat	10µg/ml Con A	Medium	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	5µg/ml $NiSO_4$	10µg/ml Con A	Medium	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
P51	48+10	2.3+1.1	N.T.	28.8+4	N.T.	3.4+0	1.6+0.4	12.2+2	49+1	2.8+1.5	28.2+2	42.4+7	29.2+2
P62	81+8	2.8+0.8	31.4+2	25.5+2	13.9+1	14+4	1.0+0.5	10.1+3	90+9	2.1+0.7	40.3+3	28.8+4	24.9+3

Results in cpm x 10^{-3} (mean + SD)

N.T. = Not Tested

Appendix VI Proliferation of PBM and Enriched T-Cells With or Without Added Plastic-Adherent Blood Cells in the Presence of Con A or Nickel Sulphate

	PBM					T			T+10% Ad				
Cont	10µg/ml Con A	Medium	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$	10µg/ml Con A	Medium	5µg/ml $NiSO_4$	10µg/ml Con A	Medium	10µg/ml $NiSO_4$	5µg/ml $NiSO_4$	2.5µg/ml $NiSO_4$
C7	82+10	0.9+0.5	N.T.	0.9+0	1.0+0	1.3+0	1.0+0.5	N.T.	11+1	1.1+0.2	N.T.	1.4+0	N.T.
C11a	23+3	3.9+1.1	4.5+0	4.9+0	3.5+0	3.3+0	1.8+0.3	1.5+0	13+0	2.2+0.3	4.9+1	3.6+1	2.5+0
C11b	37+4	6.1+0.8	14.8+2	7.8+3	7.0+1	5.1+1	3.6+0.6	5.2+0	22+6	5.1+0.8	8.9+2	6.4+1	6.0+0
C11c	33+6	3.9+1.4	11.2+2	7.8+1	4.1+1	3.5+1	1.8+0.2	0.9+0	46+5	3.9+1.3	8.3+1	8.1+1	4.3+1
C11d	30+3	3.3+0.4	12.4+2	9.4+1	7.4+1	2.7+1	1.1+0.2	1.4+0	28+6	3.4+1.3	7.9+1	5.6+1	4.9+0
C12a	18+2	4.6+0.1	6.4+0	6.1+1	N.T.	2.5+0	1.0+0.2	N.T.	17+2	3.4+0.7	N.T.	3.6+0	N.T.
C12b	20+1	2.6+0.3	6.8+1	7.2+1	N.T.	2.0+1	1.4+0.3	N.T.	13+3	1.3+0.6	1.9+0	3.6+0	N.T.
C12c	64+6	3.3+0.7	5.3+0	4.5+1	3.4+0	2.4+0	1.6+0.6	1.6+0	33+5	2.5+0.7	6.2+2	4.0+1	2.1+0
C13a	56+6	2.7+0.5	N.T.	6.3+1	N.T.	1.7+0	0.9+0.1	2.0+0	43+4	2.2+0.7	N.T.	4.3+1	N.T.
C13b	33+3	3.1+0.8	6.4+2	4.5+1	2.9+0	3.4+0	1.9+0.8	N.T.	14+4	2.1+0.6	1.2+0	2.4+1	4.7+2
C17	99+10	1.3+0.3	3.1+1	1.3+0	2.0+0	1.8+0	1.6+0.4	1.3+0	20+5	2.2+0.9	N.T.	1.5+0	N.T.
C26a	31+4	5.7+0.6	11.8+5	5.0+1	6.8+1	2.3+1	1.4+0.3	N.T.	26+5	2.8+0.3	12.7+2	7.5+1	5.7+1
C26b	38+8	3.8+0.9	N.T.	7.7+4	9.2+0	3.7+0	3.5+1.3	2.9+0	54+12	5.5+0.9	16.1+2	12.4+2	19.8+3
C26c	8+1	1.5+0.5	2.7+0	2.6+0	N.T.	1.3+0	1.5+0.3	0.9+0	6+0	1.3+0.3	N.T.	1.3+0	N.T.
C27	31+4	5.7+1.5	15.0+2	14.7+1	12.1+1	3.9+0	3.5+0.5	1.9+0	41+8	17.3+2	20.2+1	23.4+1	14.7+1
C31	55+2	3.0+0.6	1.3+0	1.1+0	3.5+1	5.5+0	3.1+1.6	5.4+0	72+4	2.4+1.1	4.6+0	3.1+0	4.4+1

Results in cpm x 10^{-3} (mean + SD) N.T = Not Tested

Appendix VII Proliferation of PBM and Enriched T-Cells with or without Added Plastic-Adherent Blood Cells or Epidermal Cells in the Presence of Con A or Nickel Sulphate

	10µg/ml Con A					Medium				
Pat	PBM	T	T+10%Ad	T+20%Ep	T+40%Ep	PBM	T	T+10%Ad	T+20%Ep	T+40%Ep
P20	27.5+2	11.5+1	19.6+4	18.8+2	37.7+8	6.3+1.5	7.3+0.8	5.5+1.1	6.5+1.5	7.2+0.8
P41	40.1+5	3.4+0	20.9+4	10.6+2	20.9+1	12.2+2.7	2.5+0.5	9.6+1.5	1.9+0.5	2.6+0.5
P46	12.5+1	4.5+1	27.8+4	56.3+12	96.3+8	3.5+1.4	2.8+1.0	3.3+0.7	2.1+0.9	3.4+1.7
P48	25.8+3	3.2+1	25.7+3	47.6+2	58.6+5	4.2+1.5	2.4+0.8	4.5+0.7	N.T.	2.0+1.0
P62	80.9+8	14.0+4	90.1+9	93.9+10	112.2+15	2.8+0.8	1.0+0.5	2.1+0.7	1.3+0.7	1.1+0.5
Pat	5µg/ml $NiSO_4$					2.5µg/ml $NiSO_4$				
P20	56.6+6	27+11	67.9+3	77.5+10	83.0+3	52.5+5	N.T.	72.3+5	72.0+1	90.5+6
P41	38.8+5	N.T.	34.4+3	N.T.	16.8+3					
P46	13.4+1	2.5+1	12.3+1	11.8+1	23.3+4	10.6+2	N.T.	10.7+2	7.5+1	23.3+4
P48	26.2+3	4.2+0	25.7+3	24.1+2	21.9+2					
P62	25.5+2	10.1+3	28.8+4	30.0+4	31.3+2	13.9+1	N.T.	24.9+3	31.3+6	29.6+2

Results in cpm x 10^{-3} (mean + SD)

N.T. = Not Tested

Appendix VIII Stimulation of PBM and Enriched T-Cells with or without Added Plastic-Adherent Blood Cells or Epidermal Cells in the Presence of Con A or Nickel Sulphate

	10µg/ml Con A					Medium				
Cont	PBM	T	T+10%Ad	T+20%Ep	T+40%Ep	PBM	T	T+10%Ad	T+20%Ep	T+40%Ep
C11	29.8+3	2.7+1	27.6+6	44.1+10	29.3+6	3.3+0.4	1.1+0.2	3.4+1.3	2.9+0.6	3.4+1.5
C12	63.6+6	2.4+0	33.0+5	81.3+0	77.3+4	3.3+0.7	1.6+0.6	2.5+0.7	7.0+0.6	7.0+2.0
C26	37.6+8	3.7+0	53.9+12	24.5+3	34.0+5	3.8+0.9	3.5+1.3	5.5+0.9	1.7+1.0	1.2+0.5
C31	55.0+2	5.5+0	72.1+4	75.9+6	87.0+12	3.0+0.6	3.1+1.6	2.4+1.1	1.8+0.4	1.2+0.5

	5µg/ml $NiSO_4$					2.5µg/ml $NiSO_4$				
Cont										
C11	9.4+1	1.4+0	5.6+1	7.6+2	8.3+1					
C12	4.5+1	1.6+0	4.0+1	N.T.	10.8+0					
C26	7.7+4	2.9+0	16.1+2	3.0+1	3.4+1	9.2+0	N.T.	12.4+2	2.0+0	N.T.
C31	1.1+0	5.4+0	3.1+0	1.8+0	0.6+0					

Results in cpm x 10^{-3} (mean + SD) N.T. = Not Tested

APPENDIX IX

Inhibition of Cell Proliferation. Pulsing with Anti-MHC II

PBM from 1 nickel-sensitive patient and 1 control were pulsed with antibodies against the MHC molecules (ie incubated with the antibodies for 1 hour at room temperature and then washed 3 times). 10µg/ml Con A (Figs a) and 5µg/ml $NiSO_4$ (Figs b) were then added to the cells and the 6 day LTT carried out. Results in cpm x 10^{-3} (Mean ± SD).

Fig IX.1
Patient's PBM + 0-2600ng/ml DA6.231 (anti-HLA-DP, -DQ, -DR)

Fig IX.2
Control's PBM + 0-2600ng/ml DA6.231

Fig IX.3
Patient's PBM +0-1100ng/ml B7/21 (anti-HLA-DP)

Fig IX.4
Control's PBM + 0-1100ng/ml B7/21

Fig IX.5
Patient's PBM + 0-1560ng/ml Leu 10 (anti-HLA-DQ)

Fig IX.6
Control's PBM + 0-1560ng/ml Leu 10

Fig IX.7
Patient's PBM + 0-800ng/ml L368 (anti-$beta_2$microglobulin)

Fig IX.8
Control's PBM + 0-800ng/ml L368.

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody DA6.231
Patient 38

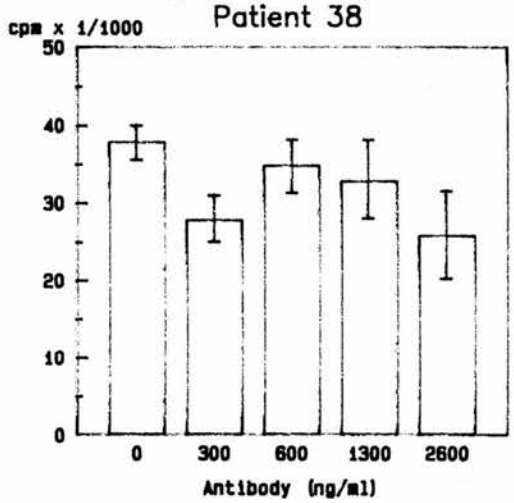


Fig IX.1a

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody DA6.231
Control 17

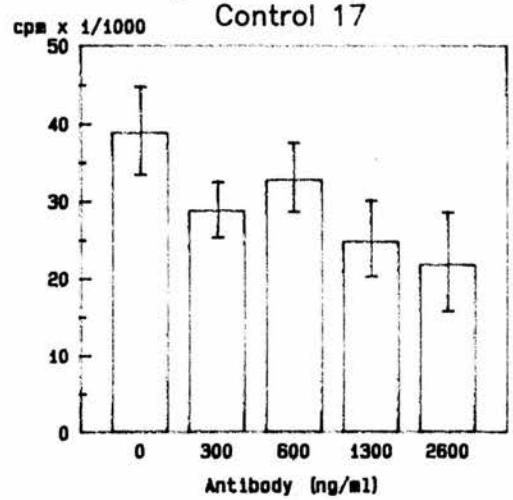


Fig IX.2a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody DA6.231
Patient 38

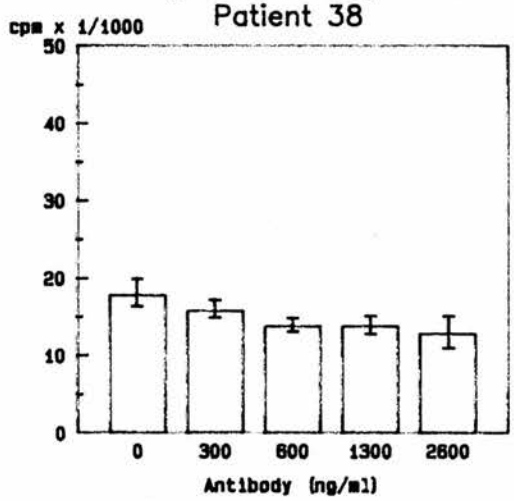


Fig IX.1b

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody DA6.231
Control 17

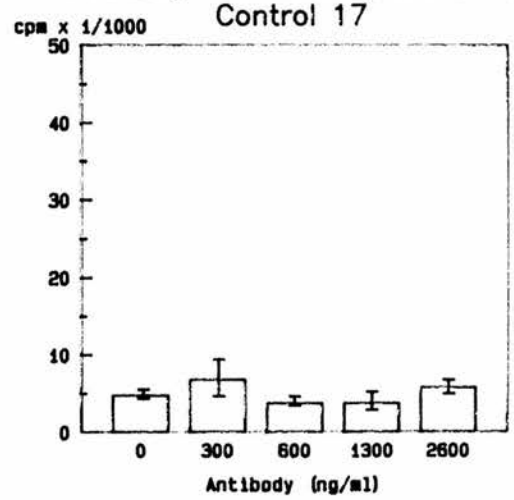


Fig IX.2b

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody B7/21
Patient 38

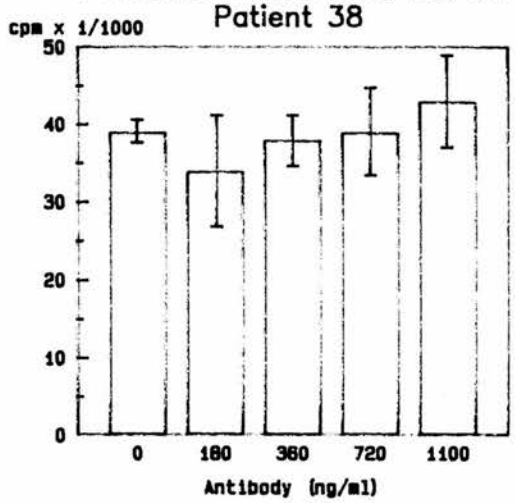


Fig IX.3a

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody B7/21
Control 17

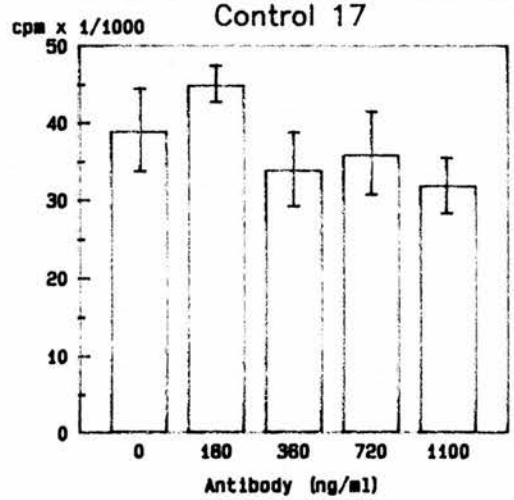


Fig IX.4a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody B7/21
Patient 38

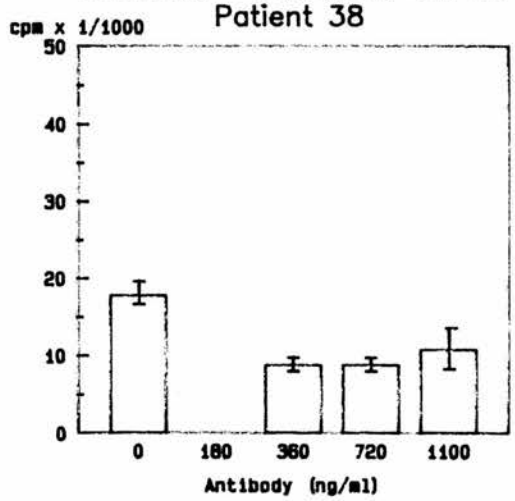


Fig IX.3b

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody B7/21
Control 17

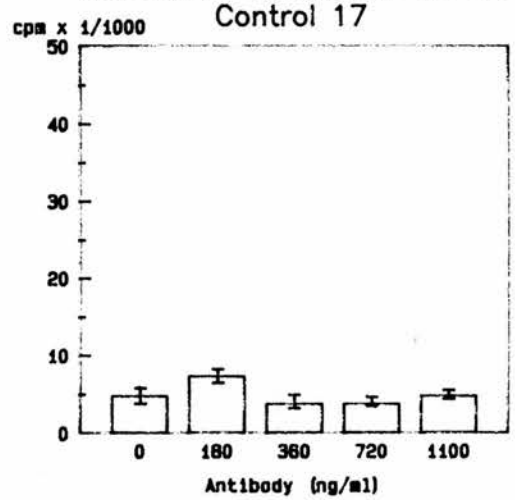


Fig IX.4b

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody Leu 10
Patient 38

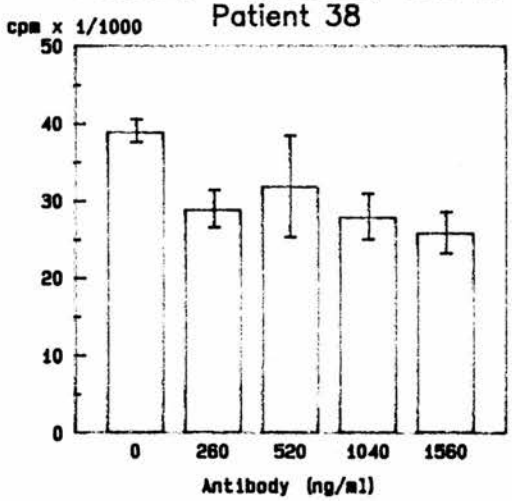


Fig IX.5a

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody Leu 10
Control 17

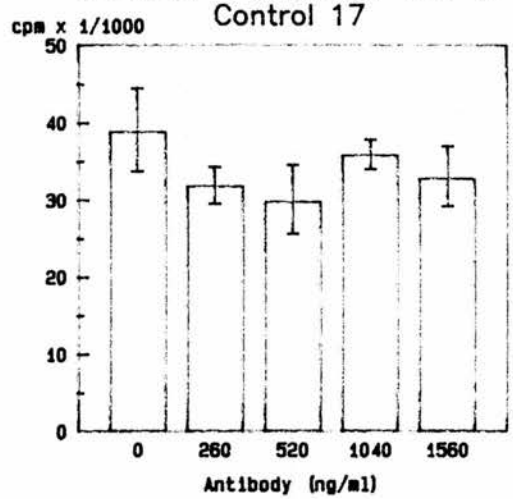


Fig IX.6a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody Leu 10
Patient 38

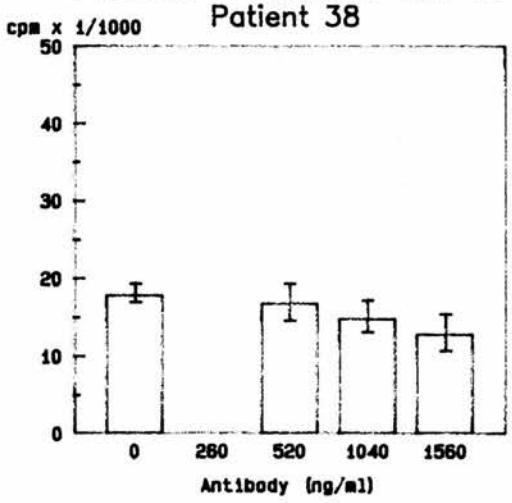


Fig IX.5b

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody Leu 10
Control 17

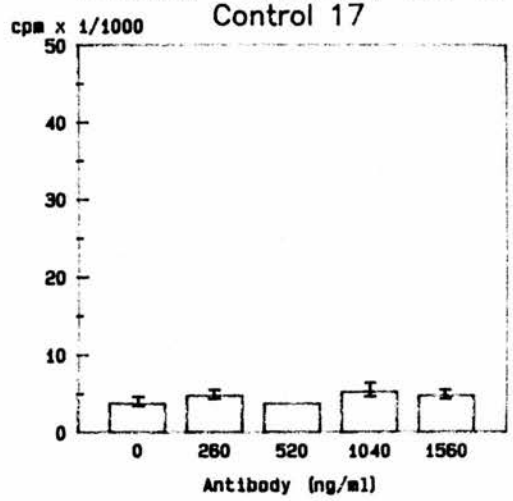


Fig IX.6b

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody L368
Patient 38

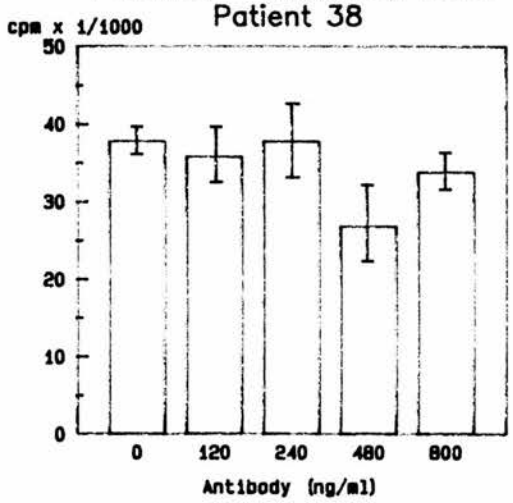


Fig IX.7a

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody L368
Control 17

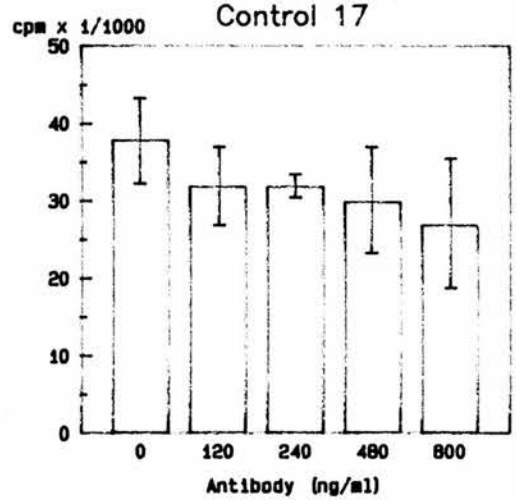


Fig IX.8a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody L368
Patient 38

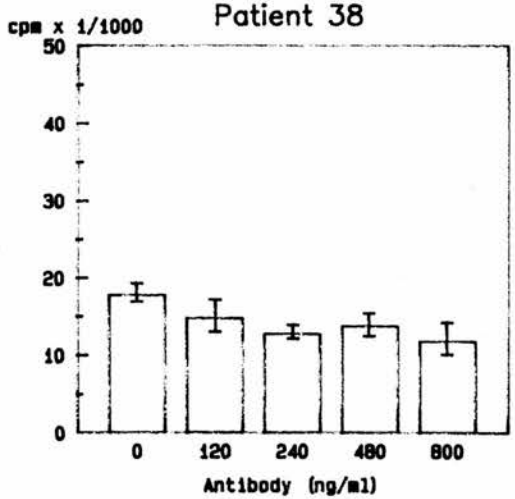


Fig IX.7b

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody L368
Control 17

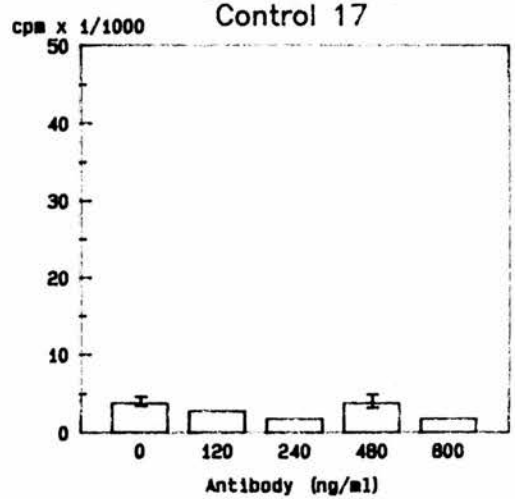


Fig IX.8b

APPENDIX X

Inhibition of Cell Proliferation. Pulsing with Anti-MHC II

PBM from 1 nickel-sensitive patient and 1 non-sensitised control were pulsed with antibodies against the MHC molecules (ie incubated with the antibodies for 1 hour at room temperature and then washed 3 times). 10µg/ml Con A (figs a) and 5µg/ml $NiSO_4$ (figs b) were then added to the cells and the 6 day LTT carried out. Results in cpm x 10^{-3} (Mean ± SD).

Fig X.1
Patient's PBM + 0-6550ng/ml DA6.231 (anti-HLA-DP, -DQ, -DR)

Fig X.2
Control's PBM + 0/6550ng/ml DA6.231

Fig X.3
Patient's PBM + 0-2100ng/ml B7/21 (anti-HLA-DP)

Fig X.4
Control's PBM + 0-2100ng/ml B7/21

Fig X.5
Patient's PBM + 0-3200ng/ml Leu 10 (anti-HLA-DQ)

Fig X.6
Control's PBM + 0-3200ng/ml Leu 10

Fig X.7
Patient's PBM + 0-2010ng/ml L368 (anti-beta$_2$microglobulin)

Fig X.8
Control's PBM + 0-2010ng/ml L368.

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody DA6.231
Patient 11

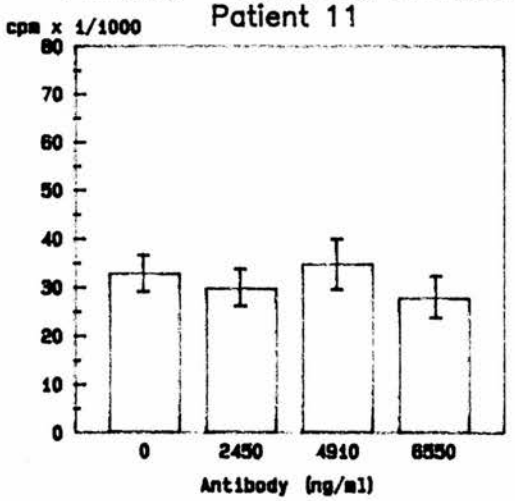


Fig X.1a

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody DA6.231
Control 11

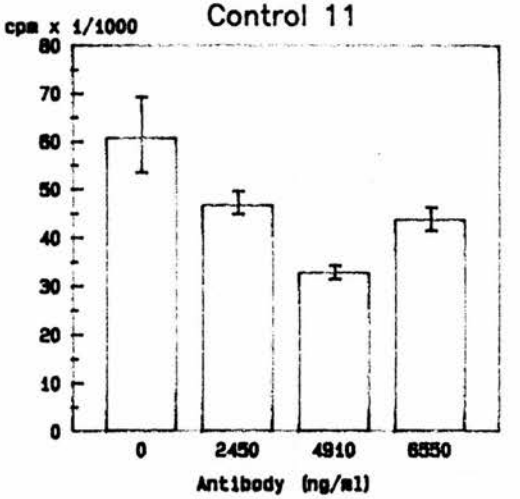


Fig X.2a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody DA6.231
Patiient 11

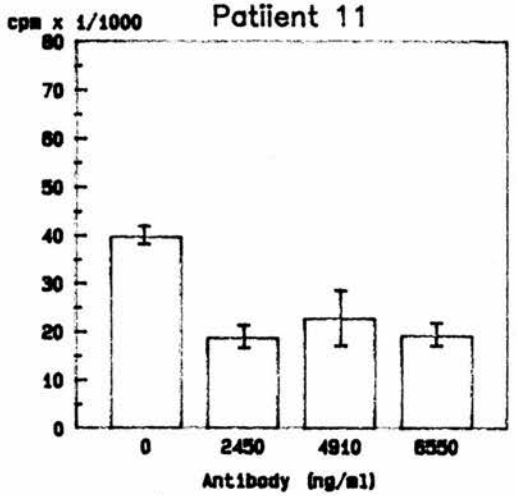


Fig X.1b

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody DA6.231
Control 11

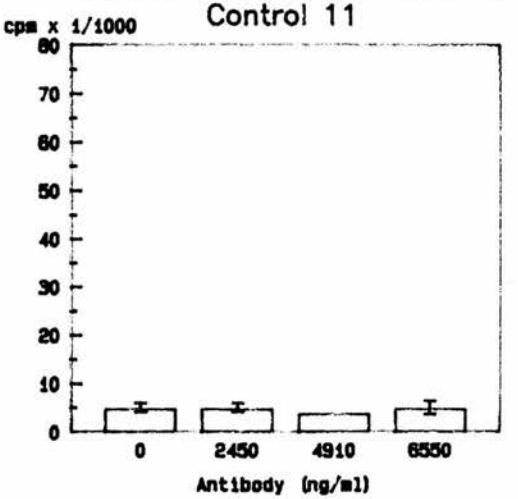


Fig X.2b

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody B7/21
Patient 11

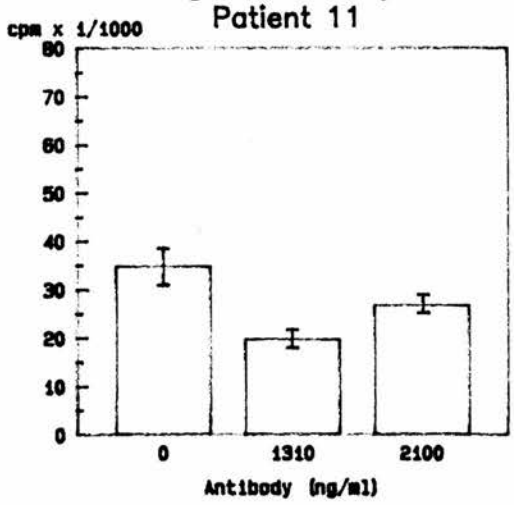


Fig X.3a

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody B7/21
Control 11

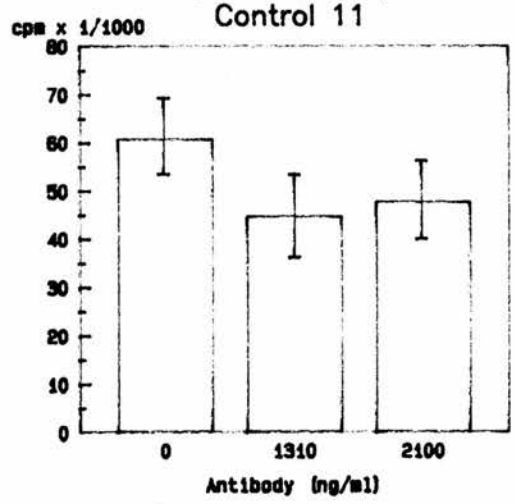


Fig X.4a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody B7/21
Patient 11

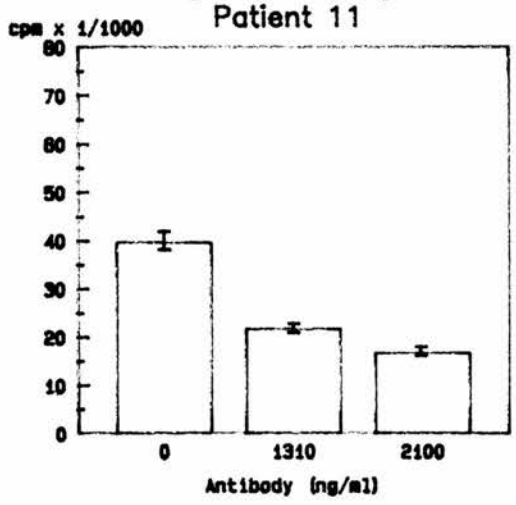


Fig X.3b

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody B7/21
Control 11

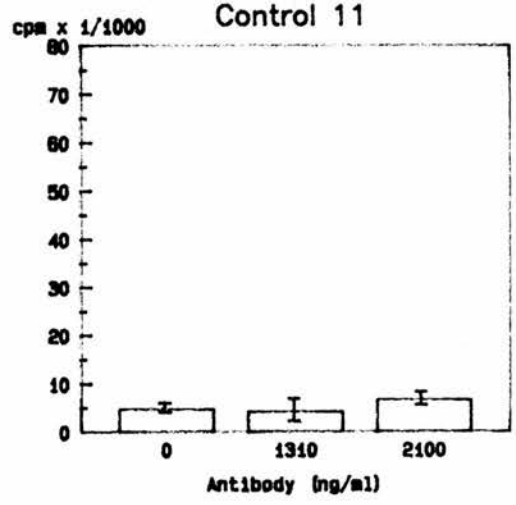


Fig X.4b

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody Leu 10
Patient 11

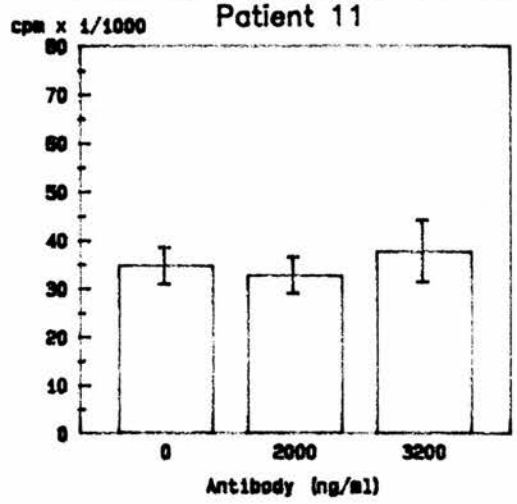


Fig X.5a

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody Leu 10
Control 11

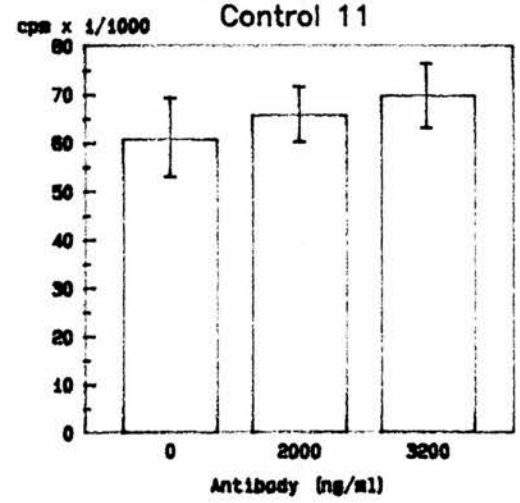


Fig X.6a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody Leu 10
Patient 11

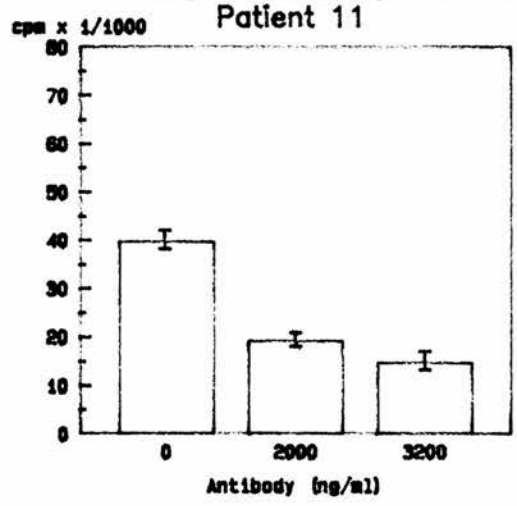


Fig X.5b

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody Leu 10
Control 11

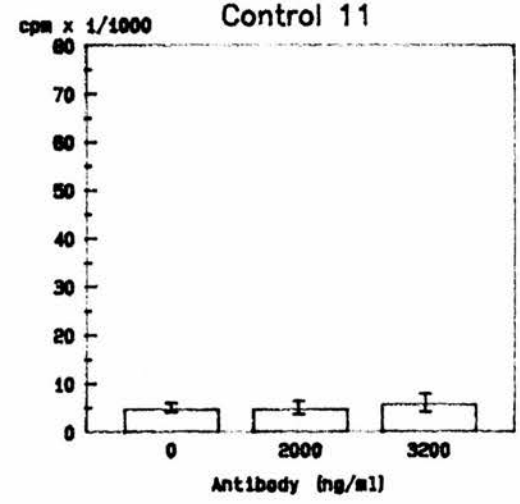


Fig X.6b

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody L368
Patient 11

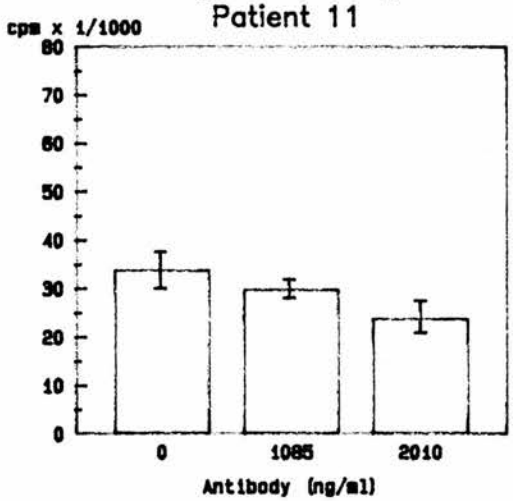


Fig X.7a

Inhibition of Con A–Induced Cell Proliferation
Pulsing - Antibody L368
Control 11

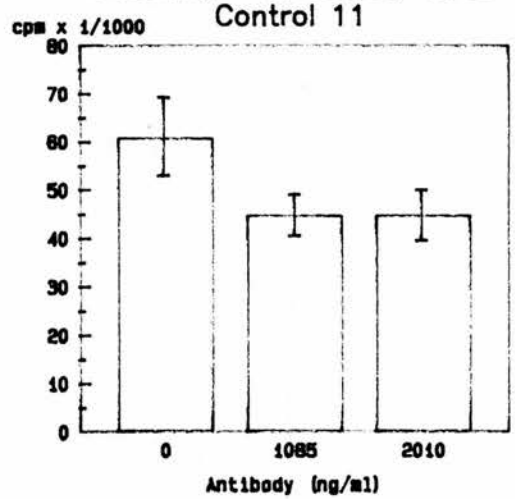


Fig X.8a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody L368
Patient 11

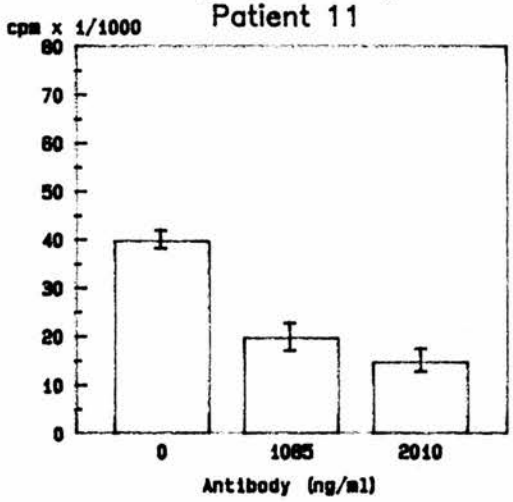


Fig X.7b

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Pulsing - Antibody L368
Control 11

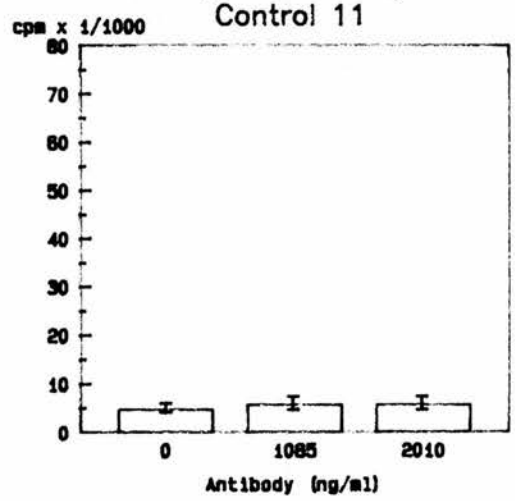
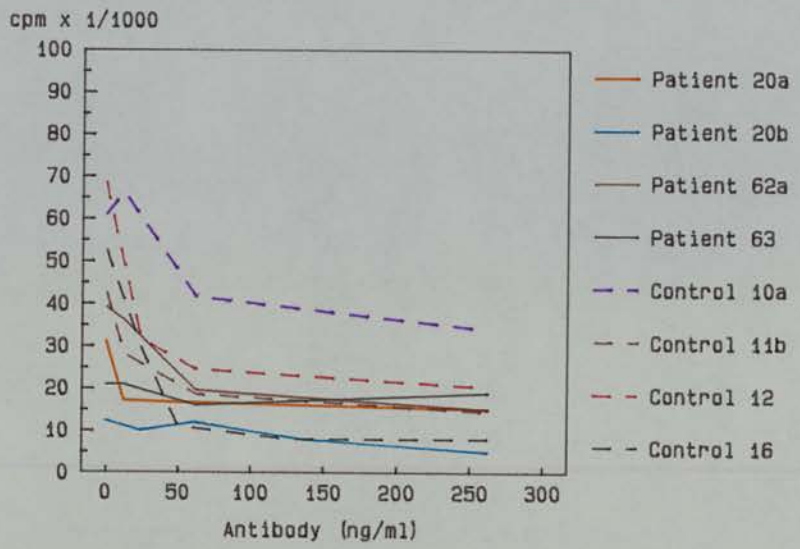


Fig X.8b

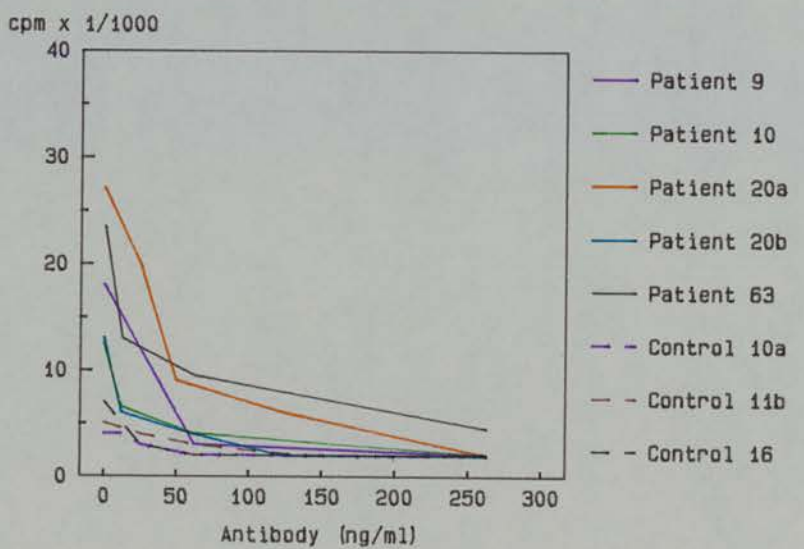
Inhibition of Con A–Induced Cell Proliferation
Antibody DA6.231 (Anti-HLA-DP, -DQ, -DR)

Fig XI.1a



Inhibition of Nickel Sulphate–Induced Cell Proliferation
Antibody DA6.231 (Anti-HLA-DP, -DQ, -DR)

Fig XI.1b



PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml DA6.231 (anti-HLA-DP, -DQ, -DR) and 10µg/ml Con A (Fig XI.Ia, 4 patients, 4 controls) or 5µg/ml $NiSO_4$ (Fig XI.Ib, 5 patients, 3 controls) in the 6 day LTT. Results in cpm x 10^{-3} (Mean).

Fig XI.2a

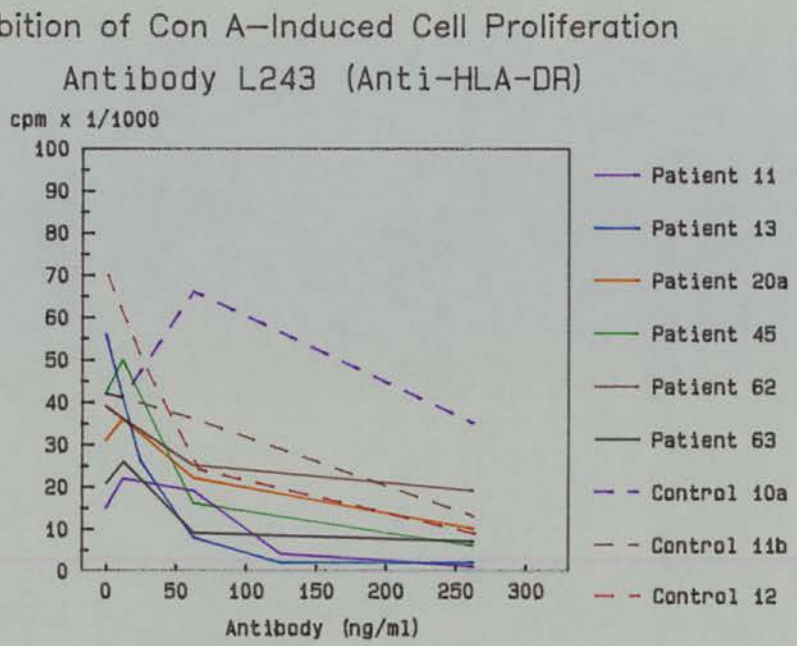
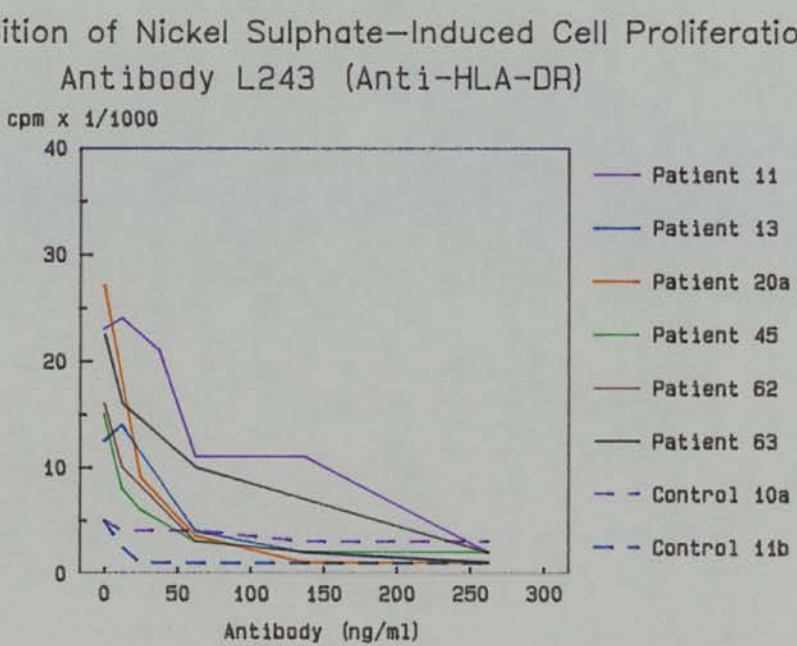


Fig XI.2b



PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml L243 (anti-HLA-DR) and 10µg/ml Con A (Fig XI.2a, 5 patients, 3 controls) or 5µg/ml $NiSO_4$ (Fig XI.2b, 6 patients, 2 controls) in the 6 day LTT. Results in cpm x 10^{-3} (Mean).

Fig XI.3a

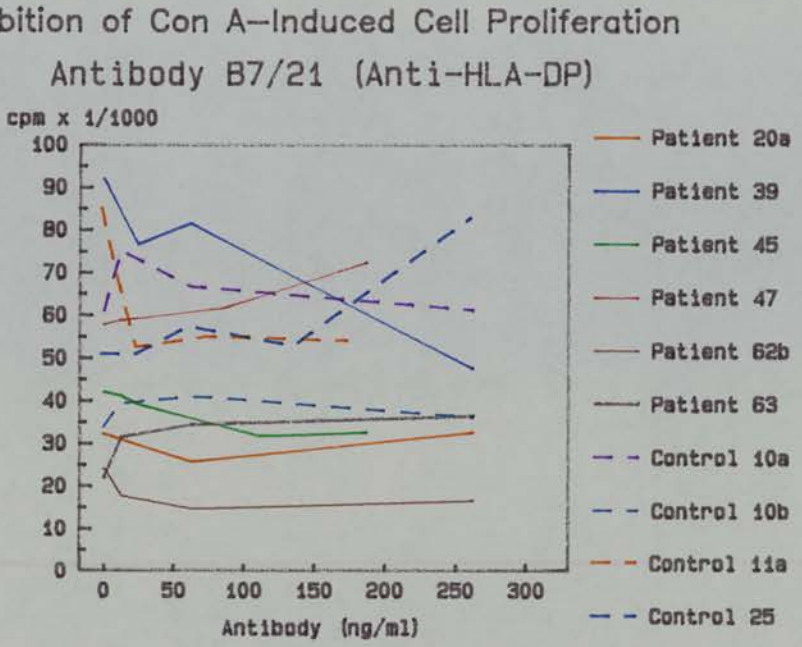
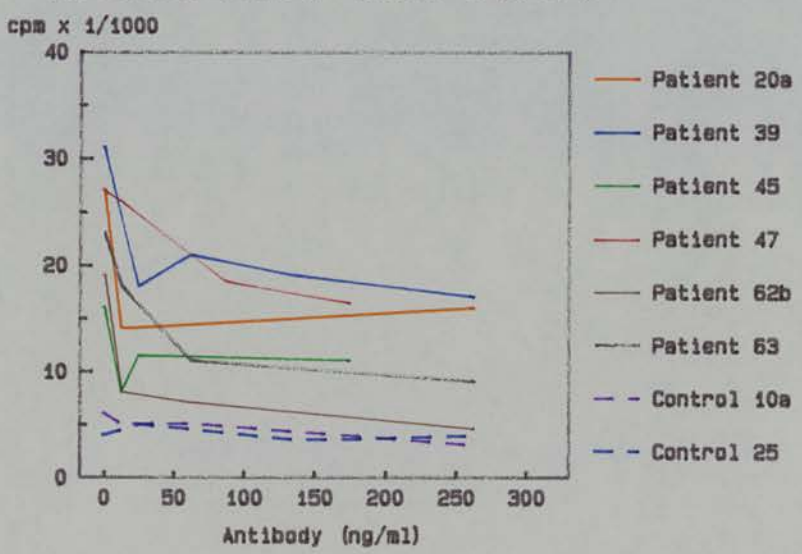


Fig XI.3b



PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml B7/21 (anti-HLA-DP) and 10µg/ml Con A (Fig XI.3a, 6 patients, 4 controls) or 5µg/ml $NiSO_4$ (Fig XI.3b, 6 patients, 2 controls) in the 6 day LTT. Results in cpm x 10^{-3} (Mean).

Fig XI.4a

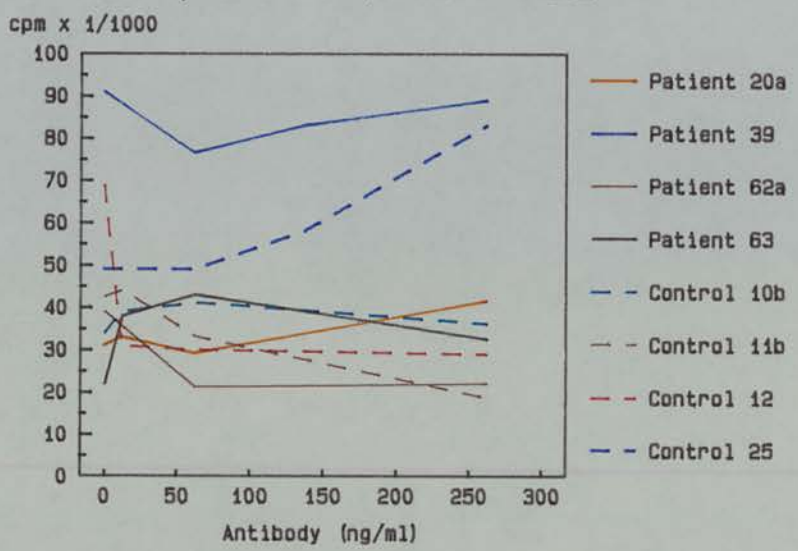
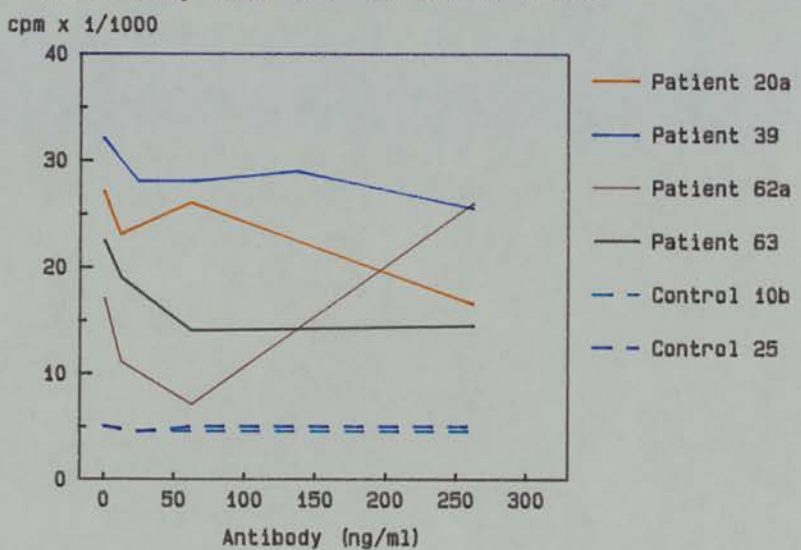


Fig XI.4b



PBM from nickel-sensitive patients and non-sensitised controls were incubated with 0-260ng/ml Leu 10 (anti-HLA-DQ) and 10µg/ml Con A (Fig XI.4a, 4 patients, 4 controls) or 5µg/ml $NiSO_4$ (Fig XI.4b, 4 patients, 2 controls) in the 6 day LTT. Results in cpm x 10^{-3} (Mean).

Inhibition of Con A–induced Cell Proliferation
Antibody L368 (Anti-β_2-Microglobulin)

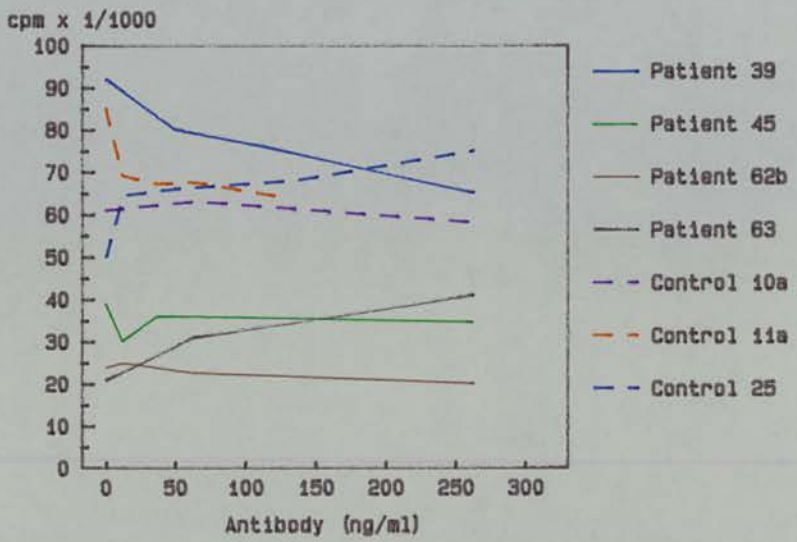


Fig XI.5a

Inhibition of Nickel Sulphate–Induced Cell Proliferation
Antibody L368 (Anti-β_2-Microglobulin)

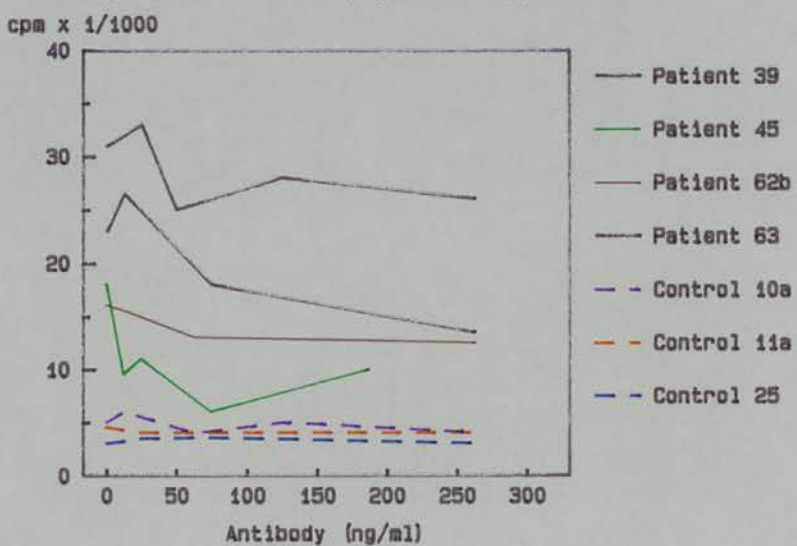


Fig XI.5b

PBM from 4 nickel-sensitive patients and 3 non-sensitised controls were incubated with 0-260ng/ml L368 (anti-beta$_2$microglobulin) and 10µg/ml Con A (Fig XI.5a) or 5µg/ml $NiSO_4$ (Fig XI.5b) in the 6 day LTT. Results in cpm x 10^{-3} (Mean).

Appendix XII Inhibition of Cell Proliferation using 260ng/ml of DA6.231 (Anti-HLA-DP, -DQ and -DR)

	PBM						T-Cells			
DA6.231	10µg/ml Con A		Medium		5µg/ml $NiSO_4$		10µg/ml Con A		Medium	5µg/ml $NiSO_4$
(ng/ml)	0	260	0	260	0	260	0	260	0	0
Pat										
P1	37+6	8.4+2	6.1+0.9	1.7+0	25.1+2	4.3+0	2.4+0	N.T.	1.6+0.5	N.T.
P11	22+2	4.1+1	6.3+0.9	2.2+0	19.0+4	2.2+0	2.0+0	N.T.	1.4+0.3	2.1+0
P51	48+10	8.8+2	2.3+1.1	1.9+0	28.8+4	2.2+0	3.4+0	N.T.	1.6+0.4	12.2+2
Cont										
C12a	18+2	4.2+1	4.6+0.1	2.3+0	6.1+1	3.9+0	2.5+0	N.T.	1.0+0.2	N.T.
C12b	20+1	8.0+1	2.6+0.3	2.7+0	7.2+1	3.9+1	2.0+1	2.8+1	1.4+0.3	N.T.
C13a	56+6	23.2+7	2.7+0.5	0.9+0	6.3+1	1.1+0	1.7+0	N.T.	0.9+0.1	2.0+0

	T-Cells + 10% Plastic-Adherent Cells					
Pat						
P1	13+1	6.6+1	3.5+0.8	2.4+0	11.4+1	7.2+1
P11	11+4	1.5+0	3.3+0.9	1.6+0	12.8+3	1.4+0
P51	49+1	13.2+4	2.8+1.5	1.9+0	42.4+7	2.2+0
Cont						
C12a	17+2	9.9+1	3.4+0.7	2.7+0	3.6+0	2.1+0
C12b	13+3	10.1+1	1.3+0.6	2.4+1	3.6+0	2.1+0
C13a	43+4	22.2+3	2.2+0.7	1.5+0	4.3+1	0.7+0

N.T. = Not Tested

Results in cpm x 10^{-3} (mean + SD)

Appendix XIII Inhibition of Cell Proliferation using 260ng/ml and 52ng/ml of L243 (Anti-HLA-DR)

	PBM						T-Cells						
L243	10µg/ml Con A			Medium			5µg/ml $NiSO_4$			10µg/ml Con A		Medium	5µg/ml
(ng/ml)	0	260	52	0	260	52	0	260	52	0	260	0	$NiSO_4$
Pat													
P20a	28+3	7.2+1	15.2+2	5.4+1.5	2.0+0	2.7+0	27.9+2	4.5+2	18.4+2	3.5+1	3.1+1	2.9+0.9	1.5+0
P40	39+5	8.1+0	N.T.	4.1+0.7	2.3+0	N.T.	43.9+7	5.9+3	N.T.	2.8+0	N.T.	1.2+0.6	N.T.
P48a	34+3	17.2+3	25.7+2	5.1+0.7	4.1+2	4.7+1	33.7+3	4.1+1	9.7+2	7.8+4	2.6+0	4.3+1.6	20.9+5
P50	98+6	16.4+2	35.0+2	4.3+1.6	1.6+0	6.3+1	17.5+3	7.4+3	10.1+1	7.3+2	2.9+0	3.6+1.1	6.4+1
Cont													
C12d	33+6	6.1+0	20.9+2	3.9+1.4	3.9+1	3.7+0	7.8+1	1.7+0	3.7+1	3.5+1	1.2+0	1.8+0.2	0.9+0
C13b	33+3	13.2+2	N.T.	3.1+0.8	1.6+0	N.T.	4.5+1	1.7+0	N.T.	3.4+0	N.T.	1.9+0.8	N.T.
C27	31+4	12.7+3	23.6+3	5.7+1.5	2.9+1	3.6+1	14.7+1	1.4+0	4.1+2	3.9+0	4.7+2	3.5+0.5	1.9+0

	T-Cells + 10% Plastic-Adherent Cells								
Pat									
P20a	19+2	6.5+0	9.2+1	4.4+1.1	2.0+0	1.9+1	39.7+4	3.9+1	17.3+3
P40	29+8	4.8+1	N.T.	4.6+1.1	3.6+0	N.T.	73.1+6	3.0+1	N.T.
P48a	26+3	8.5+1	17.2+2	4.1+1.0	1.9+0	2.5+0	34.1+1	2.6+1	10.9+2
P50	60+6	14.5+1	26.1+2	4.4+0.7	3.2+1	1.7+0	26.6+5	5.0+1	6.6+2
Cont									
C12d	46+5	7.5+0	19.5+4	3.9+1.3	1.6+0	1.6+0	8.1+1	1.3+0	1.8+0
C13b	14+4	3.5+1	N.T.	2.1+0.6	1.9+0	N.T.	2.4+1	2.2+1	N.T.
C27	41+8	12.1+2	20.5+3	17.3+2	2.9+0	2.0+0	23.4+1	1.9+0	3.9+1

N.T. = Not Tested

Results in cpm x 10^{-3}

(mean + SD)